

8070 8080 8090 8100 8110
 GGGACGCAGG GGGTGGGAAG CCCTCAAATA TTGGTGGAAT CTCCTACAGT

 8120 8130 8140 8150 8160
 ATTGGAGTCA GGAAGTAAAG AATAGTGCTG TTAGCTTGCT CAATGCCACA

 8170 8180 8190 8200 8210
 GCCATAGCAG TAGCTGAGGG GACAGATAGG GTTATAGAAG TAGTACAAGG

 8220 8230 8240 8250 8260
 AGCTTGTAGA GCTATTCGCC ACATACCTAG AAGAATAAGA CAGGGCTTGG

 8270 8280
 AAAGGATTTT GCTATAAGA.

REMARKS

Applicants respectfully request reconsideration and reexamination of this application.

The specification has been amended to correct contradictory information given regarding the file history of this application, as requested by the Examiner.

Additionally, claim 12 has been cancelled, without prejudice, in response to the Examiner's Restriction Requirement. Claim 12 has been cancelled for the sole purpose of advancing the prosecution of this application, and applicants reserve the right to prosecute the subject matter of claim 12 in a related application.

Finally, claim 11 has been amended to recite a "nucleic acid" encoded by a recited list of nucleotide sequences, as requested by the Examiner. As the foregoing amendments do not introduce new matter, it is respectfully requested that they be entered by the Examiner.

Applicants acknowledge the election of Group I, claim 11, drawn to nucleic acid having various HIV-1 sequences, with traverse. It is courteously submitted that a search of the subject matter of Groups 1 and 2 would not be burdensome. Accordingly, the withdrawal of the Restriction Requirement is respectfully requested.

Claim 11 was rejected under 35 U.S.C. § 101 because the claimed invention allegedly lacks patentable utility and as disclosed is allegedly inoperative. Applicants respectfully traverse this ground for rejection.

The Examiner stated that although the specification sets forth the nucleic acids of HIV-1 claimed by applicants, the specification "does not demonstrate a utility for these nucleotide sequences." See page 4, lines 2-4 of Paper No. 7. Applicants respectfully disagree.

Applicants' claimed nucleic acids can be used as diagnostic tools to detect the presence or absence of an HIV-1 infection in a biological sample. See page 14, line 11 through page 15, line 8 of the specification.

The Examiner's statements regarding utility first addressed the effectiveness of the claimed nucleic acids in diagnostic methods to detect HIV-1:

[a]pplicant has not demonstrated a utility for these sequences as probes. How specific are they for detecting HIV and distinguishing it from other retroviruses, in particular HTLV I and II?

See page 4 of the Paper No. 7.

HIV-1 is a unique retrovirus. With the use of cloned probes, no hybridization is detected between HIV-1 and HTLV-I or -II. See Alizon et al., "Molecular Cloning of Lymphadenopathy-Associated Virus," Nature, 312:757-760 at page 760 (1984) (Exhibit 1); and Wain-Hobson et al., "Nucleotide Sequence of the AIDS Virus, LAV," Cell, 40:9-17 at pages 14-15 (1985) (Exhibit 2). HIV-1 is both morphologically and biochemically distinct from HTLV-I and -II. See Barre-Sinoussi et al., "Isolation of a T-Lymphotropic Retrovirus From a Patient at Risk for Acquired Immune Deficiency Syndrome (AIDS)," Science, 220:868-871 at page 868 (1983) (Exhibit 3). Accordingly, one of skill in the art would not expect applicants' claimed nucleic acids corresponding to ORFs of HIV-1 to detect HTLV-I or HTLV-II in a biological sample.

Secondly, the Examiner stated that the claimed invention lacks utility as applicants have only identified open reading frames, or "ORFs," of HIV-1, and have not disclosed any function of the claimed nucleotide sequences. See page 4 of Paper No. 7. Applicants respectfully disagree.

As noted above, applicants have identified several nucleic acids which correspond to open reading frames ("ORFs") of HIV-1. If the genetic code is read in nonoverlapping triplets, there are three possible ways of translating a nucleotide sequence into protein, depending on the starting point. These are called "reading frames." All of the claimed nucleic acids

corresponding to ORFs of HIV-1 may not translate into proteins of HIV-1. However, this does not mean that they are not useful. For example, applicants teach that the disclosed nucleic acids are useful in hybridization assays to detect the presence of HIV-1 in a biological sample or blood-related product. See page 14, line 11 through page 15, line 8 of the specification. In a hybridization assay, labelled nucleic acids according to the present invention are used to detect RNA or proviral DNA of HIV-1 in, for example, a biological sample. See e.g., Hames et al., eds., Nucleic Acid Hybridization: A Practical Approach, 30-38 and 185-187 (IRL Press, Washington, DC, 1985) (Exhibit 4). Thus, the claimed nucleic acids have utility as probes in a diagnostic assay to detect the presence of HIV-1 nucleic acid.

Finally, applicants note that all that is required to demonstrate enablement for the claimed nucleic acids is some utility. See E.I. du Pont de Nemours & Co. v. Berkley & Co., 205 U.S.P.Q. 1, at page 10, footnote 17 (8th Cir. 1980) ("A small degree of utility is sufficient. The claimed invention must only be capable of performing some beneficial function An invention does not lack utility merely because the particular embodiment disclosed in the patent lacks perfection or performs crudely Nor is it essential that the invention accomplish all its intended functions . . . partial success being sufficient to demonstrate patentable utility . . .") (Citations ommitted; emphasis in original) (Exhibit 5). See also Envirotech Corp. v. Al George, Inc., 221 U.S.P.Q. 473, 480

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(Fed. Cir. 1984) ("the defense of non-utility cannot be sustained without proof of total incapacity.") (Exhibit 6).

In the present case, applicants have identified nucleic acids encoding ORFs of HIV-1 which are useful to detect the presence of an HIV-1 nucleic acid. For example, the probes are useful for detecting an HIV-1 infection in a biological sample. This usefulness satisfies the "some" utility standard set forth by the court in E.I. du Pont de Nemours. Accordingly, applicants respectfully request the withdrawal of this ground for rejection.

Claim 11 was rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention. Applicants respectfully traverse this ground for rejection.

The Examiner stated that claim 11 should be directed to "nucleic acids," rather than "nucleotide sequences." As applicants have made the requested amendment to claim 11, this ground for rejection is moot.

Applicants respectfully request reconsideration and reexamination of this application at the Examiner's convenience.

The Commissioner is hereby authorized to charge any fees associated with this Amendment to our Deposit Account

No. 06-0916. If a fee is required for an Extension of Time under 37 C.F.R. § 1.136 not accounted for above, such extension is requested and should also be charged to our Deposit Account.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER

By: Michele M. Schafer
Michele M. Schafer
Reg. No. 34,717

Dated: November 24, 1993

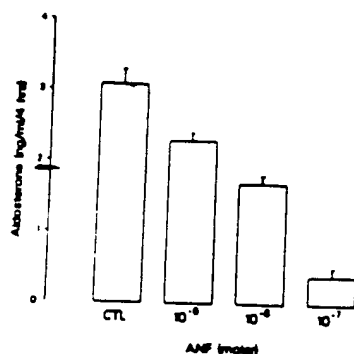


Fig. 1 Effect of ANF(8-33) on basal aldosterone secretion. Rat glomerulosa cells were prepared by enzymatic digestion of 20 rat adrenals after enucleation. The cells remaining on the capsule were digested for 30 min with a mixture of collagenase and DNase (4 mg ml⁻¹, 4 µg ml⁻¹) for 30 min. Dispersed cells were filtered through gauze and centrifuged at 800 r.p.m. for 15 min. The pellet was resuspended in M199 buffer containing 0.1% bovine serum albumin (BSA) and the cells centrifuged at 800 r.p.m. for 15 min. The cell pellet was again resuspended in M199-0.1% BSA buffer and distributed in 900-µl aliquots to 12 × 75 plastic tubes. The samples were preincubated for 90 min in a 37 °C waterbath under an atmosphere of 5% CO₂/95% O₂. Aliquots of the test samples were added in a 100 µl volume and incubated for 4 h. Aldosterone and corticosterone were measured by radioimmunoassay using antisera purchased from Endocrine Sciences, Oxnard, California, and ³H-labelled steroid from NEN. Results are the mean ± s.e.m. of seven replicates. Statistical analysis was performed by analysis of variance and all points are significant (*P* < 0.01) from control. (ANF(8-33) was the gift of Drs R. Hirschmann and D. F. Veber of Merck, Sharp and Dohme Research Laboratories).

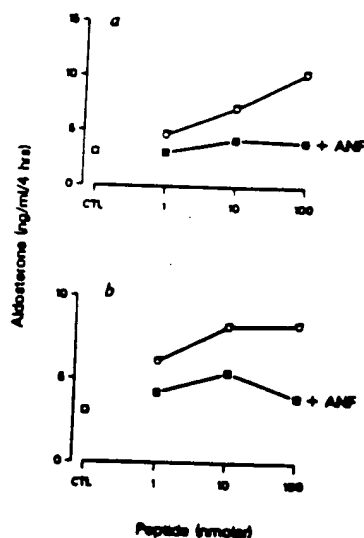


Fig. 2 Effect of ANF(8-33) on stimulated aldosterone secretion. **a**, Rat glomerulosa cells were prepared as described in Fig. 1 and incubated with synthetic human ACTH either alone (open circles) or in combination with equimolar amounts of ANF(8-33) (closed squares). Aldosterone secretion was measured as above by radioimmunoassay. **b**, Cells were incubated with synthetic angiotensin-II (AN-II) either alone (open circles) or in the presence of equimolar amounts of ANF(8-33) (closed squares). Control cells received neither peptide, thereby indicating the ability of ANF to decrease aldosterone production to basal levels. Results are the mean ± s.e.m. of 7 replicates and all points are significant when compared with their respective control (*P* < 0.001). Synthetic hACTH(1-39) and angiotensin II were synthesized by Dr Nicholas Ling by solid-phase methodology.

(8-33) as a natriuretic hormone, and now in inhibiting basal and stimulated aldosterone formation, suggests that its biological activities are an integral part of the homeostatic mechanisms regulating sodium retention. Furthermore, unlike somatostatin, its inhibitory effect is not restricted to angiotensin-stimulated aldosterone secretion, but affects the formation of both basal and stimulated mineralocorticoids. Moreover, at no point was ANF(8-33) observed to stimulate aldosterone. The observations reported here provide the groundwork for defining the mechanisms by which atrial-derived peptides affect sodium retention and suggest that this peptide may be responsible for the attenuated effects of AN-II on the adrenal cortex during sodium loading^{16,17}. The understanding of some clinical forms of idiopathic hypo- and hypertension^{18,19} may therefore result from defining the interactions between ANF, the adrenal cortex and the basic mechanisms regulating ANF secretion.

After submission of this manuscript, Chartier *et al.*²⁰ and DeLean *et al.*²¹ reported findings similar to those reported here.

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Molecular cloning of lymphadenopathy-associated virus

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Lymphadenopathy-associated virus (LAV) is a human retrovirus first isolated from a homosexual patient with lymphadenopathy syndrome, frequently a prodrome or a benign form of acquired immune deficiency syndrome (AIDS)¹. Other LAV isolates have subsequently been recovered from patients with AIDS or pre-AIDS²⁻⁴ and all available data are consistent with the virus being the causative agent of AIDS. The virus is propagated on activated T lymphocytes and has a tropism for the T-cell subset OKT4 (ref.

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6), in which it induces a cytopathic effect. The major core protein of LAV is antigenically unrelated to other known retroviral antigens^{1,2,7}. LAV-like viruses have more recently been independently isolated from patients with AIDS and pre-AIDS. These viruses, called human T-cell leukaemia/lymphoma virus type III (HTLV-III)⁸⁻¹¹ and AIDS-associated retrovirus (ARV)¹², seem to have many characteristics in common with LAV and probably represent independent isolates of the LAV prototype. We have sought to characterize LAV by the molecular cloning of its genome. A cloned LAV complementary DNA was used to screen a library of recombinant phages constructed from the genomic DNA of LAV-infected T lymphocytes. Two families of clones were characterized which differ in a restriction site. The viral genome is longer than any other human retroviral genome (9.1–9.2 kilobases).

The cDNA first-strand of LAV was synthesized in an endogenous, detergent-activated reaction. LAV virions were purified from the supernatant of FR8 cells, a B-lymphoblastoid LAV-producing line¹³, and the reaction was primed with oligo(dT). Three cDNA clones, pLAV13, 75 and 82, carrying inserts of 2.5, 0.6 and 0.8 kilobases (kb), respectively, were characterized further (Fig. 1). All three inserts have a common restriction pattern at one end, indicative of a common priming site. The 50-base pair (bp) common *Hind*III–*Pst*I fragment was sequenced and shown to contain an oligo(dA) stretch preceding

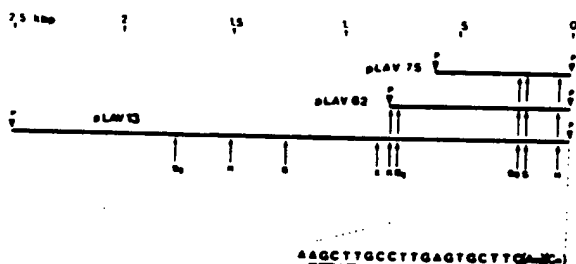


Fig. 1 Restriction maps of cDNA clones derived from LAV genomic RNA. Restriction sites: B, *Bam*HI; Bg, *Bgl*II; H, *Hind*III; K, *Kpn*I; S, *Sac*I; X, *Xba*I.

Methods: LAV cDNA was synthesized in an endogenous detergent-activated reaction. For each reaction, LAV virions were purified on a 20–60% sucrose gradient as described previously¹, from 200 ml of supernatant of the LAV-producing FR8 line¹³. Virus-containing fractions were pooled, diluted with NTE buffer (100 mM NaCl, 10 mM Tris-HCl pH 7.8, 1 mM EDTA) and centrifuged (Beckman type SW56 rotor, 50,000 r.p.m., 60 min). The viral pellet was resuspended in 250 µl of NTE. Reaction volume was adjusted to 1 ml and final concentrations were: 50 mM Tris-HCl pH 7.8, 25 mM NaCl, 6 mM MgCl₂, 10 mM dithiothreitol, 0.02% Triton X-100, 0.1 mM of each of dATP, dGTP, TTP, 4 µM dCTP including 200 µCi of [α -³²P]dCTP (400 Ci mmol⁻¹, Amersham) and 50 µg ml⁻¹ oligo(dT) primer. Incubation was at 37 °C. After 15 min, dCTP was added to 25 µM. At 45 min, the reaction was stopped with EDTA and SDS (final concentrations 20 mM and 0.5%, respectively). After 1 h of proteinase K digestion (100 µg ml⁻¹, 37 °C), the reaction mixture was extracted with phenol/chloroform and cDNA-RNA hybrids were ethanol-precipitated. Second-strand synthesis with nuclease-free DNA polymerase I (Boehringer) and RNase H (BRL) and dC-tailing with terminal transferase (Boehringer) were performed according to Gubler and Hoffman²⁰. Tailed double-stranded cDNA was annealed to dG-tailed *Pst*I-linearized pBR327 vector. *Escherichia coli* C600 recBC was transformed by the CaCl₂ method; 500 recombinant clones were screened *in situ*²¹ with a ³²P-labelled LAV cDNA in which the first strand had been synthesized as described above, except that an alkaline hydrolysis step was included. Approximately 10% of recombinants proved positive, the majority of which formed a family of cross-hybridizing clones. Three recombinants, pLAV13, pLAV75 and pLAV82, carrying inserts of 2.5, 0.6 and 0.8 kb, respectively, were analysed further. There are no sites for *Eco*RI, *Nru*I, *Pvu*I, *Sal*I, *Sma*I, *Sna*I or *Xba*I in the pLAV13 insert. The *Hind*III–*Pst*I fragment was subcloned into M13mp8 and sequenced according to Sanger *et al.*²² using a 15-mer primer (Biolabs) and [α -³²P]dCTP (Amersham).

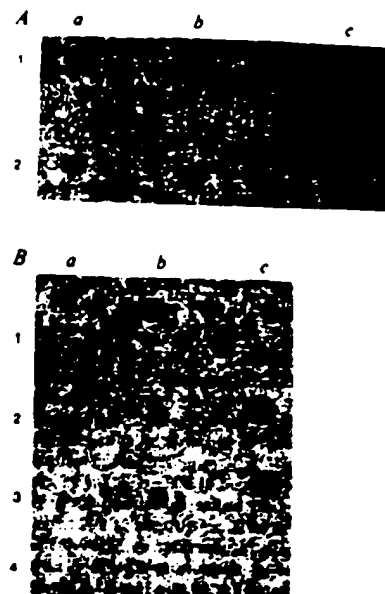


Fig. 2 Rapid dot-blot technique for LAV detection in cell culture supernatant. Spots represent: A, a, 1 µl; b, 2 µl; c, 4 µl of concentrated (250 ×) cell culture supernatant from (1) LAV-producing CEM cells (reverse transcriptase activity (RT), determined as described previously¹, was 140,000 c.p.m. ml⁻¹); (2) LAV-producing Epstein-Barr-transformed B-cell line FR8 (RT 175,000 c.p.m. ml⁻¹); B, a, 1 µl; b, 2 µl; c, 5 µl of 100 × concentrated supernatant from (1) uninfected normal T lymphocytes (no RT activity); (2) LAV-producing normal T lymphocytes (RT 170,000 c.p.m.); (3) LAV-producing CEM line (RT 150,000 c.p.m.); and (4) culture of bone marrow lymphocytes from a haemophilic patient with AIDS (RT 7,000 c.p.m.).

Methods: Cell culture supernatants were pelleted through 0.5 ml 20% sucrose cushions in NTE buffer (Beckman type SW56 rotor, 50,000 r.p.m., 1 h, 4 °C). The pellet was resuspended in NTE buffer as indicated. Concentrated virus was spotted onto dried nylon filters (Zetabind) presoaked in 20 × SSC (3 M NaCl, 0.3 M sodium citrate). After baking (at least 30 min at 80 °C), filters were hybridized with ³²P nick-translated pLAV13 insert (Fig. 1) (specific activity > 10⁶ c.p.m. per µg) for 12–16 h in stringent conditions (50% formamide, 5 × SSC, 42 °C), washed (0.1 × SSC, 0.1% SDS, 65 °C, 2 × 30 min), and exposed for 20 h (Kodak XAR5 film with an intensifying screen) at –70 °C.

the cloning dC tail. The clones are thus copies of the 3' end of a poly(A) RNA.

The specificity of pLAV13 was determined in a series of filter hybridization experiments using nick-translated pLAV13 insert as a probe. First, using an adapted spot-blot technique, we could detect LAV virion RNA from normal T cells, FR8 and other B-cell lines and CEM cells (L.M. and R. Weiss, unpublished results; Fig. 2). LAV was also detected in a bone marrow cell culture (Fig. 2B, line 4) from a haemophilic with AIDS⁴, in spite of the low titre of virus in the supernatant. Uninfected cultures proved negative (Fig. 2B, line 1). Second, the probe detected DNA in the Southern blots of LAV-infected T lymphocytes and CEM cells (Fig. 3). No hybridization was detected in DNA from uninfected lymphocytes or from normal liver (data not shown) in the same hybridization conditions. A characteristic 1.45-kb *Hind*III fragment which co-migrated with an internal viral fragment in *Hind*III-cleaved pLAV13 (Fig. 1) was detected in the Southern blots. Bands at 2.3 and 6.7 kb were also detected. Together, these data show that pLAV13 DNA is exogenous to the human genome and detects both RNA and integrated DNA forms derived from LAV-infected cells. Thus, pLAV13 is LAV specific. Being oligo(dT)-primed, pLAV13 must contain the R and U3 regions of the long terminal repeat (LTR) as well as

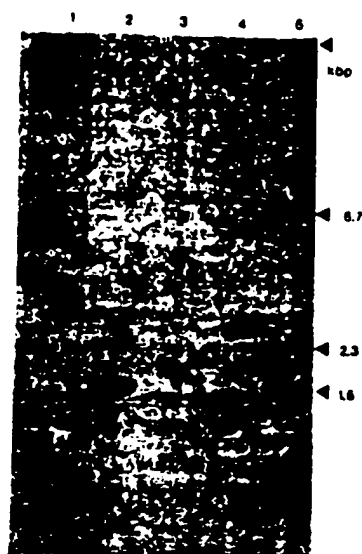


Fig. 3 Southern blot of *Hind*III-restricted genomic DNA from LAV-infected and uninfected cells hybridized with pLAV13. *Hind*III-restricted high relative molecular mass DNA from: lane 1, uninfected CEM cells; lane 2, LAV-infected CEM cells; lane 3, uninfected T cells after 5 days' culture; lane 4, LAV-infected T cells 2 days after infection; lane 5, LAV-infected T cells 5 days after infection.

Methods: Peripheral blood T lymphocytes of a healthy donor were stimulated for 3 days with phytohemagglutinin, after which they were infected with LAV (isolate BRU-LAVI) at 10^6 c.p.m. reverse transcriptase activity per 10^6 cells as described previously¹, except for part of the culture kept uninfected for controls. Two and five days after infection, genomic DNA was extracted. *Hind*III-digested DNA (10 μ g) was electrophoresed through a 0.8% agarose gel and Southern blotted. The filter was hybridized in 10 ml of 50% formamide, 5 \times SSC, 1 \times Denhardt's, 10% dextran sulphate with 100 μ g ml⁻¹ denatured sonicated salmon sperm DNA and 2 \times 10^6 c.p.m. of nick-translated pLAV13 insert (4 \times 10^4 c.p.m. per μ g) for 10 h at 42 °C. The filter was washed at 68 °C in 0.1 \times SSC, 0.1% SDS for 2 \times 30 min and exposed to Kodak XARS film at -70 °C for 16 h using an intensifying screen.

the 3' end of the coding region, assuming a conventional retroviral genome structure.

Having found a *Hind*III site about 20 bp 5' of the poly(A) stretch and thus within the R region of the LTR, we cloned the LAV genome by making a partial *Hind*III digest of genomic DNA from LAV-infected T cells of a healthy donor. A 9 \pm 1.5-kb DNA-containing fraction was precipitated and ligated into the *Hind*III arms of phage vector λ L47.1 (ref. 14). When nick-translated pLAV13 insert was used as a probe to screen $\sim 2 \times 10^6$ phage plaques *in situ*, five independent clones were obtained. A restriction map of clone λ J19 and of a *Hind*III variant, λ J81, are shown in Fig. 4. Recombinants λ J27, λ J31 and λ J57 have the same *Hind*III map as λ J19, while λ J81 is so far unique. As the two clones were derived from the first isolate¹ of LAV reported (isolate BRU, or LAVI), we refer to the two viral genomes as LAVIa (λ J19) and LAVIb (λ J81). λ J19 shows four *Hind*III bands of 6.7, 1.45, 0.6 and 0.52 kb, the first two of which correspond to bands in the genomic blot of *Hind*III-restricted DNA (Fig. 3, lane 5). The smallest bands (0.6 and 0.52 kb) were not seen in the genomic blot, but the fact that they appear in all the independently derived clones analysed indicates that they represent internal and not junction fragments, assuming random integration of LAV proviral DNA. However, the 0.52-kb band hybridizes with pLAV13 DNA (Fig. 4) through the small *Hind*III-*Pst*I fragment of pLAV13. Thus, the 0.5-kb *Hind*III fragment of λ J19 contains the R/U5 junction within the LTR. The finding of two small *Hind*III fragments in the 5' region reinforces

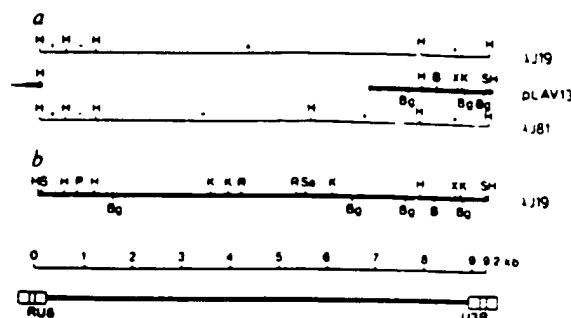


Fig. 4 Restriction maps of LAV proviral DNA in clones λ J19 (LAVIa) and λ J81 (LAVIb). a, *Hind*III restriction maps of LAV proviral DNA in clones λ J19 and λ J81. Those *Hind*III fragments detected by pLAV13 are marked by +, those not, by -. The restriction map of the pLAV13 cDNA clone is also shown. b, Restriction map of λ J19. Restriction sites: B, *Bam*HI; Bg, *Bgl*II; H, *Hind*III; K, *Kpn*I; P, *Pst*I; R, *Eco*RI; S, *Sac*I; Sa, *Sal*I; X, *Xho*I. Beneath the scale is a schema for the general structure of retroviruses showing the LTR elements U3, R and U5. Only the R/U5 boundary has been defined (Fig. 1) and other boundaries are drawn only figuratively.

Methods: DNA from LAV-infected T cells was partially digested with *Hind*III and fractionated on a 5–40% sucrose gradient in 10 mM Tris-HCl pH 8, 10 mM EDTA, 1 M NaCl (Beckman type SW41 rotor, 16 h, 40,000 r.p.m.). A single fraction (9 \pm 1.5 kb) was precipitated with 20 μ g ml⁻¹ dextran T40 as carrier and taken up in TE buffer (10 mM Tris-HCl pH 8, 1 mM EDTA). λ L47.1 (ref. 14) *Hind*III arms were prepared by first ligating the cos sites followed by *Hind*III digestion and fractionation through a 5–40% sucrose gradient as above. Fractions containing only the λ *Hind*III arms were pooled, precipitated and taken up in TE buffer. Ligation of arms to DNA was made at ~ 200 μ g ml⁻¹ DNA using a 3:1 molar excess of arms and 300 U of T4 DNA ligase (Biolabs). *In vitro* packaging lysates were made according to ref. 29. After *in vitro* packaging, the phage lysate was plated out on NM538 or a C600 recBC strain. Approximately 2×10^6 plaques were screened by *in situ* hybridization¹⁰ using nitrocellulose filters. Hybridization was performed at 68 °C in 1 \times Denhardt's solution, 0.5% SDS, 2 \times SSC, 2 mM EDTA. Probe: ³²P nick-translated insert of pLAV13 at $> 10^6$ c.p.m. per μ g. Filters were washed for 2 \times 30 min in 0.1 \times SSC 10.1% SDS at 68 °C, and exposed to Kodak XAR-5 film for 24–40 h with intensifying screens at -70 °C. Seven positive clones were identified and plaque-purified on a C600 recBC strain. Liquid cultures were grown and the recombinant phages banded in CsCl. Phage DNA was extracted and digested in the appropriate conditions. The restriction maps were orientated by hybridizing blots to pLAV13 DNA, which maps the 3' coding sequences of the viral genome as well as the U3-R region of the LTR. All cloning and amplification of LAV genomic clones was carried out in a P3 laboratory.

the usefulness of cloning LAV by partial restriction of genomic DNA.

λ J81 seems to be a restriction site polymorph of λ J19, showing five *Hind*III bands of 4.3, 2.3, 1.45, 0.6 and 0.52 kb (Fig. 4). The 2.3-kb band is readily detected in the genomic blot by a pLAV13 probe, although the 4.3-kb fragment is not. The finding that nick-translated λ J19 DNA hybridizes to all five *Hind*III bands of λ J81 in stringent hybridization and washing conditions indicates that λ J81 is a *Hind*III variant and not a recombinant virus. Also, other mapped restriction sites in λ J81 are identical to those of λ J19 (not shown). Thus, the *Hind*III restriction pattern in the Southern blot can be explained by variation within the single isolate of LAV used to infect the T cells.

HTLV-I¹⁵ and HTLV-II¹⁶ constitute a pair of C-type transforming retroviruses with a tropism for the T-cell subset, OKT4. Both genomes (comprising one LTR) are ~ 8.3 kb long^{17,18}, have an X region and show extensive sequence homology. They hybridize between themselves in reasonably stringent conditions (40% formamide, 5 \times SSC) and the X regions hybridize even at 60% formamide¹⁹. Thus, a conserved X region is a hallmark of

this class of virus. We have compared cloned LAV DNA and cloned HTLV-II DNA (pMO)²⁰ by blot-hybridization and find no cross-hybridization in low stringency conditions of hybridization and washing ($T_m = 55^\circ\text{C}$), even after 2 days' exposure at -70°C using intensifying screens (data not shown).

The human T-lymphotropic retroviruses HTLV-III⁸ and ARV¹², recently isolated from patients with AIDS or pre-AIDS, have similar morphological, biochemical and immunological properties to LAV, which suggests that they probably represent different isolates of the LAV prototype. DNA hybridization between HTLV-III and HTLV-I and -II has been reported, most noticeably at the *gag-pol* junction and less so in the characteristic X region of HTLV-I and -II²¹. As mentioned above, we could detect no such hybridization and conclude that the reported homology must have been due to either (1) the use of an uncloned cDNA as hybridization probe, (2) the fact that the isolates in question differ substantially from those we have cloned, or (3) the possibility that HTLV-III and a HTLV-I/II-like virus were co-infecting the cells. The last possibility may also apply to the preliminary report of cross-hybridization between a LAV-like virus and a cloned HTLV-II DNA probe⁷. Thus, we find no molecular evidence of a relationship between LAV and HTLV. Furthermore, the LAV genome is ~9 kb long, compared with 8.3 kb for the HTLV viruses^{17,18}. Despite their comparable genome sizes, LAV does not cross-hybridize with Visna virus²² (~9 kb) (data not shown) or with several human endogenous viral genomes (ref. 23 and M. Martin, personal communication) in non-stringent conditions ($T_m = 55^\circ\text{C}$). These data and morphological and immunological dissimilarities^{1,2} between LAV and the HTLV-I/-II pair all point to LAV being a novel class of human retrovirus.

In conclusion, we have molecularly cloned the complete genome of LAV from freshly infected activated T cells of a healthy donor. It has been shown that the tropism of certain retroviruses resides in the LTR^{24,25} and that sequence differences and insertions/deletions are present in the LTRs of leukaemogenic and non-leukaemogenic retroviruses. It is thus possible that LAV and LAV-like viruses passaged through B- and T-transformed cell lines^{9,12,13} might have undergone some attenuation. Although the cDNA clones were made from a LAV-producing B-cell line, the genomic clones were isolated from LAV-infected normal T cells. Thus, the clones represent LAV genomes that have not been selected or adapted to a particular cell line. However, the LAV genome is shown to be polymorphic even within a single isolate and independent isolates will probably differ widely.

The availability of cloned LAV DNA should facilitate the understanding of the molecular mechanism of viral replication, and the tropism of the virus. The DNA sequence of LAV opens up the possibility of expressing the viral *gag* and *env* gene products and of studying the molecular basis of LAV antigenicity.

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Molecular cloning of AIDS-associated retrovirus

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Retroviruses cause a wide variety of diseases in avian and mammalian species. Human acquired immune deficiency syndrome (AIDS) leads to collapse of the immune system and death by a wide variety of opportunistic infections; unusual forms of cancer are associated with this syndrome. Retroviruses have been recovered from tissues of AIDS patients and from patients with related conditions. These similar newly-isolated viruses are lymphadenopathy-associated virus (LAV)¹, human T-cell lymphotropic virus (HTLV-III)^{2,3} and AIDS-associated retrovirus (ARV-2)⁴. We have identified a RNA genome of ~9 kilobases (kb) in viruses purified from the culture medium of a human T-cell tumour line infected with ARV-2. A cDNA probe made from viral RNA detected circular DNA molecules and proviral forms in infected cells. We prepared a library of infected cell DNA. Recombinant phage included those with a 9.5-kb proviral DNA and viral DNA permuted with respect to the single *EcoRI* site. Comparison of three ARV isolates from different AIDS patients revealed polymorphism of restriction endonuclease sites.

HUT-78 cells, originating from a human T-cell lymphoid tumour⁵, were used to propagate the ARV-2 strain of virus⁶. To characterize the viral genome, RNA was extracted from purified virions and electrophoresed on agarose gels containing methyl mercury hydroxide⁷. A distinct ~9-kb RNA species was observed (Fig. 1) with smaller heterogeneous RNA and some ribosomal RNA species. The 9-kb RNA species was used as a template with random primers in a reverse transcriptase reaction to produce a virus-specific cDNA probe⁷. RNA of virus obtained from cells infected with ARV-2 or with two additional isolates, ARV-3 and ARV-4, showed distinct bands at 9 kb that hybridized with the cDNA probe (Fig. 1).

With this cDNA probe, we examined the structure of viral DNA in infected cells by digestion with restriction enzymes, electrophoresis in agarose gels and Southern blotting. No specific bands were detected in several digests of DNA from uninfected cells (Fig. 2a, lanes C, E), whereas bands were seen in infected cells (Fig. 2a, lane A). Undigested DNA from infected cells contained a species at 5.5 kb, a faint species at 6 kb

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Nucleotide Sequence of the AIDS Virus, LAV

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Summary

The complete 9193-nucleotide sequence of the probable causative agent of AIDS, lymphadenopathy-associated virus (LAV), has been determined. The deduced genetic structure is unique: it shows, in addition to the retroviral gag, pol, and env genes, two novel open reading frames we call Q and F. Remarkably, Q is located between pol and env and F is half-encoded by the U3 element of the LTR. These data place LAV apart from the previously characterized family of human T cell leukemia/lymphoma viruses.

Introduction

The recent onset of severe opportunistic infections among previously healthy male homosexuals has led to the characterization of the acquired immune deficiency syndrome (AIDS) (Gottlieb et al., 1981; Masur et al., 1981). The disease has spread dramatically, and new high-risk groups have been identified: patients receiving blood products, intravenous drug addicts, and individuals originating from Haiti and Central Africa (Piot et al., 1984). AIDS is a fatal disease, and there is at present no specific treatment. The causative agent was suspected to be of viral origin since the epidemiological pattern of AIDS was consistent with a transmissible disease, and cases had been reported after treatment involving ultrafiltered anti-hemophilia preparations (Daly and Scott, 1983). A decisive step in AIDS research was the discovery of a novel human retrovirus called lymphadenopathy-associated virus (LAV) (Barré-Sinoussi et al., 1983). The properties of the virus consistent with its etiological role in AIDS are: the recovery of many independent isolates from patients with AIDS or related diseases (Montagnier et al., 1984); high LAV seropositivity among these populations (Brun-Vézinet et al., 1984); a tropism and cytopathic effect in vitro for the helper/inducer T-lymphocyte subset T4 (Klatzmann et al., 1984), also found depleted in vivo.

Other groups have reported the isolation of human retroviruses, the human T cell leukemia/lymphoma/lymphotropic virus type III (HTLV-III) (Popovic et al., 1984) and the AIDS-associated retrovirus (ARV), which display biological and sero-epidemiological properties very similar to if not identical with those of LAV (Levy et al., 1984; Popovic et al., 1984; Schüpbach et al., 1984). Both LAV and HTLV-

III genomes have been molecularly cloned (Alizon et al., 1984; Hahn et al., 1984). Their restriction maps show remarkable agreement, including a Hind III restriction site polymorphism, bearing in mind the variability of this virus (Shaw et al., 1984) and confirming that these two viruses represent a single viral lineage.

In addition to its obvious diagnostic and therapeutic potential, the LAV DNA nucleotide sequence is essential to an understanding of the genetics and molecular biology of the virus and its classification among retroviruses. We report here the complete 9193-nucleotide sequence of the LAV genome established from cloned proviral DNA.

Results

DNA Sequence and Organization of the LAV Genome

We have reported previously the molecular cloning of both cDNA and integrated proviral forms of LAV (Alizon et al., 1984). The recombinant phage clones were isolated from a genomic library of LAV-infected human T-lymphocyte DNA partially digested by Hind III. The insert of recombinant phage λ J19 was generated by Hind III cleavage within the R element of the long terminal repeat (LTR). Thus each extremity of the insert contains one part of the LTR. We have eliminated the possibility of clustered Hind III sites within R by sequencing part of an LAV cDNA clone, pLAV 75 (Alizon et al., 1984), corresponding to this region (data not shown). Thus the total sequence information of the LAV genome can be derived from the λ J19 clone.

Using the M13 shotgun cloning and dideoxy chain termination method (Sanger et al., 1977), we have determined the nucleotide sequence of λ J19 insert. The reconstructed viral genome with two copies of the R sequence is 9193 nucleotides long. The numbering system starts at the cap site (see below) of virion RNA (Figure 1).

The viral (+) strand contains the statutory retroviral genes encoding the core structural proteins (gag), reverse transcriptase (pol), and envelope protein (env), and two extra open reading frames (orf) that we call Q and F (Table 1). The genetic organization of LAV, 5'LTR-gag-pol-Q-env-F-3'LTR, is unique. Whereas in all replication-competent retroviruses pol and env genes overlap, in LAV they are separated by orf Q (192 amino acids) followed by four small (<100 triplets) orf. The orf F (206 amino acids) slightly overlaps the 3' end of env and is remarkable in that it is half-encoded by the U3 region of the LTR.

Such a structure clearly places LAV apart from previously sequenced retroviruses (Figure 2). The (-) strand is apparently noncoding. The additional Hind III site of the LAV clone λ J81 (with respect to λ J19) maps to the apparently noncoding region between Q and env (positions 5166-5745). Starting at position 5501 is a sequence (AAGCCT) that differs by a single base (underlined) from the Hind III recognition sequence. It is anticipated that many of the restriction site polymorphisms between different isolates will map to this region.

GCTGTCTCTGTTAGACACGATTTCGCCCTGGGAGCTTCTTGCCATACTAGGCACCCTACTGCTTAAGCCTCAATAAAGCTTGCGTTGAAGTGTCTGCTGCCCTGCTGTGT

100
GTGACTCTGGTAACTAGACAGATCCCTCAGACCCCTTTTAGTCACTGTGAAAAAATCTCTAGCACTGGGCCCCAACAGCGGCTTGAAGCGAAAAGCAAACACAGAGCCTCTCTCGACGCA

200
GAG - LeuAlaGluAlaArgArgArgGluLeuPheValAlaSerValLeuSer

CACTCGGCTTGCTGAAGCGGCGCACGGCAAGACGGCGGCGGCGACTGCTGACTACGCCAAAAATTTTGAAGCGGCGTACAAGGAGAGATGGGTGGCAGCGCTCAGTATTAA

300
GlyGlyGluLeuAspArgTrpGluLysIleArgLeuArgProGlyGlyLysLysLysTyrLysLeuHisIleValTrpAlaSerArgGluGluGluArgPheAlaValAsnProGly

CCC GG G G A A T T A G A C T C G A T G C G G A A A A A T T C G T T A A G C C A G G G G A A G A A A A A T A T A A T T A A A C A C T A T A G T T G C C A A C G G G A C T G A C A A G T T C G C A A T T C G C A T T A A T C T G

400
LeuLeuGluThrSerGlnGlyCysArgGlnIleLeuGlyGlnLeuGlnProSerLeuGluThrGlySerGluThrArgSerLeuTyrAsnThrValAlaThrLeuTyrCysValHis

CGCTGTGTAAGAACAATCAGAAGGCTGAGACAAATACTGGGACGGCTACAAACCTCCCTTACAGACGATGACAGAACTTAGATCATTTATATAACAGTACGACCAACCTCTATTGTGTGC

500
GlnArgIleGluIleLysAspThrLysGluAlaLeuAspLysIleGluGluGluGlnAsnLysSerLysLysLysAlaGlnGlnAlaAlaAspThrGlyHisSerSerGlnValSer

ATCAAGGATAGAGATAAAGACACCAAGGAAGCTTTAGACACAGATAGAGGAACGACAAAAAGTAAAGAAAAAGCACAGCAAGCAGCAGCTGACACAGGACGACAGCAGCGCACTCA

600
GlnAsnTyrProlleValGlnAsnIleGlnGlyGlnMetValHisGlnAlaIleSerProArgThrLeuAsnAlaTrpValLysValValGluGluLysAlaPheSerProGluValIle

GCCAAAATTTACCTTATAGTCGACAACATCCAGGGGCAATTCGTACATCAGGCCATATCACTAGAAGTTTAAATGCATGGTAAAGTAGTAGAAGAGAAAGCTTTTACGCCCAAGATGCA

700
ProMetPheSerAlaLeuSerGluGlyAlaThrProGlnAspLeuAsnThrMetLeuAsnThrValGlyGlyHisGlnAlaAlaMetGlnMetLeuLysGluThrIleAsnGluAla

TACCAATGTTTTTACGATTATCAGAAGGAGCCACCCCAACAGATTTAAACACCATGCTAAACACAGCTGGGGGACATCAAGCAGCCATGCAAAATGTTAAAGACACCATCAANTGACGAAG

800
AlaGluTrpAspArgValHisProValHisGlaGlyProlleAlaProGlyGlnMetArgGluProArgGlySerAspileAlaGlyThrThrLeuGlnGlnIleGlyTrp

CTGCAGCAATGGGTAGACTGATGCTACGATGTCAGCGGCTTTCAGCAGCGGATGACAGAACCAAGGGGAAGCATAGGACGAACTACTAGTACCTTACGACCAAAATAGGAT

900
MetThrAsnAsnProProlleProValGlyGluIleTyrLysArgTrpIleIleLeuGlyLeuAsnLysIleValArgMetTyrSerProThrSerIleLeuAspileArgGlnGlyTrp

CGATGACAAATAATCCACCTATCCAGTAGGAGAAATTTATAAAGATGGATAATCTGGGATTAATAAAATAGTAAGATGTATAGCCCTACCGCATCTTGGACATAAGACAAGAC

1000
LysGluProPheArgAspTyrValAspArgPheTyrLysThrLeuArgAlaGluGlnAlaSerGlnGluValLysAsnTrpMetThrGluThrLeuLeuValGlnAsnAlaAsnProAsp

CAAAAGACCCCTTTAGAGACTATGACAGCGGTCTTATAAACTCTAAGAGCCGAGCAAGCTTCAGGAGGTAAAAAATTTGGATGACAGAACTCTTGGTGC AAAATGCCAACCCAG

1100
CysLysThrIleLeuLysAlaLeuGlyProAlaAlaThrLeuGluGluMetMetThrAlaCysGlnGlyValGlyGlyProGlyHisLysAlaArgValLeuAlaGluAlaMetSerGln

ATTGTAAGACTATTTTAAAGCATTTGGGACCGACGCTACACTAGAGAAATGATGACAGCATGTGCGGAGCTGGGAGCGCCGCCATAAGCCAAGATTTTGGCTGAAGCAATGAGCC

1200
ValThrAsnSerAlaThrIleMetMetGlnArgGlyAsnPheArgAsnGlnArgLysIleValLysCysPheAsnCysGlyLysGluGlyHisIleArgGlnAsnArgAlaProArg

AAGTACAAATTCAGCTACCATATGATGCGAAGAGGCAATTTTAGGAACCAAGAAAGAACTGTTTAAAGTCTTCAATGTCGCAAGAGGCGCATAGCAGCAAAATTCGACGGGCCCTA

1300
POL - PhePheArgGluAspLeuAlaPheLeuGlnGlyLysAlaArgGluPheSer

LysLysGlyCysTrpLysCysGlyLysGluGlyHisGlnMetLysAspCysThrGluArgGlnAlaAsnPhelGlyLysIleTrpProSerTyrLysGlyArgProGlyAsnPheLeu

CGAAAAGCGGCTGTTCGAATGTCGAAAGGAGGACACCAATGAAGATTTGACTGAGACAGGCTAAATTTTTAGGGAAGATCTGGCTTCTTACAAAGGAGGCCAGCGGAATTTTC

1400
SerGluGlnThrArgAlaAsnSerProThrArgArgGluGluGlnValTrpGlyArgAspAsnAsnSerLeuSerGluAlaGlyAlaAspArgGlnGlyThrValSerPheAsnPhePro

GlnSerArgProGluProThrAlaProGluGluSerPheArgSerGlyValGluThrThrProSerGlnGlnGlnProlleAspLysGlyLysGluLeuTyrProLeuThrSerLeu

TTACAGCAGCAGCAGAGCCCAACAGCCGCCACCAAGAGAGCTTCAGCTCTGGGTAGACACAACCTCCCTCTCAGAACGAGCGCCGATAGACAAGGAATCTATCTTTAACTTCCC

1500
GlnIleThrLeuTrpGlnArgProLeuValThrIleLysIleGlyGlyGlnLeuLysGluAlaLeuLeuAspThrGlyAlaAspAspThrValLeuGluGluMetSerLeuProGlyArg

ArgSerLeuPheGlyAsnAspProSerSerGln

TCAGTCACTCTTTGGCAACGACCCCTGCTCAATAAAGATAGGGGGCACTAAAGGAAGCTCTATTAGATACAGAGCAGATGATACAGTATTAGAAGAAATGAGTTTCCGAGGAAG

1600
TrpLysProTyrMetIleGlyGlyIleGlyPheIleLysValArgGlnTyrAspGlnIleLeuIleGluIleCysGlyHisLysAlaIleGlyThrValLeuValGlyProThrPro

ATGGAAACCAAAATGATAGGGGAAATGGAGGTTTTATCAAGTAAGACAGATGATACAGATACTCATAGAAATCTCTGGACATAAGCTATAGGTACAGTATTAGTACGACTACACC

1700
ValAsnIleIleGlyArgAsnLeuLeuThrGlnIleGlyCysThrLeuAsnPheProlleSerProIleGluThrValProValLysLeuLysProGlyMetAspGlyProLysValLys

TGTCAACATAATGGAAGAAATCTGTGACTCAGATGCTGTCACCTTAAATTTCCCATTTAGTCTCTATTGAACATGATACAGTAAAAATTAAGCCAGCAATGGATGGCCCAAAAGTTAA

1800
GlnTrpProLeuThrGluGluLysIleLysAlaLeuValGluIleCysThrGluMetGluLysGluGlyLysLysSerLysIleGlyProGluAsnProTyrAsnThrProValPheAla

ACAATGGCCATTGACAGAGAAAAATAAAGCATTTAGTAAAAATTTGACAAAATGGAAGGAAGCGGAAATTTCAAAAATTTGGGCTGAAAATCCATACAATACTCCAGTATTTC

1900
IleLysLysLysAspSerThrLysTrpArgLysLeuValAspPheArgGluLeuAsnLysArgThrGluAlaGlnAspPheThrGlnGlnGlyIleProHisProAlaGlyLeuLysLys

CATAAAGAAAAAGACAGACTAAATGAGAAAAATTAGTACATTTACAGAACTTAATAAGCAACTCAAGACTTCTGGGAAGTTCAATTAGCAATACCATCCCGGCAAGTTAAAAAA

2000
LysLeuSerValThrValLeuAspValGlyAspAlaTyrPheValProLeuAspGluAspPheArgLysTyrThrAlaPheThrIleProSerIleAsnAsnGluThrProGlylle

CAAAAAATTCAGTCACTAGCTGGATGTGGGTGATGATATTTTACGTTCCCTTAGATGAAGACTTCAGGAAGTATAGTGCATTTACCATACCTAGTATAAACAATGAGACACCGGAT

2100
ArgTyrGlnTyrAsnValLeuProGlnGlyTrpLysGlySerProAlaIlePheGlnSerSerMetThrLysIleLeuGluProPheArgLysGlnAsnProAspileValIleTyrGln

TAGATATCAGTACAATGTGCTTCACAGGGATGGAAGGATCACCAGCAATATTCCAAAGTACATGACAAAAATCTTAGAGCCCTTTAGAAAACAAAATCCAGACATAGTTATCTAACA

2200
TyrMetAspAspLeuTyrValGlySerAspLeuGluIleGlyGlnHisArgThrLysIleGluGluLeuArgGlnHisLeuLeuArgTrpGlyLeuThrThrProAspLysLysHisGln

ATACATGGATGATTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGACAAAAATAGAGAGCTCAGACAACATCTGTCAGGCTGGGCACTTACCACCCAGACAAAAACATCA

2300
LysGluProProPheLeuTrpMetGlyTrpGluLeuHisProAspLysTrpThrValGlnProlleValLeuProGluLysAspSerTrpThrValAsnAspileGlnLysGlnLeuGlu

GAAGAAGCTCCATCTCTTGGATGGGTATGCACTTCATCTGATAAATGACACGCTGCTAGCTGCTGCCAAAAAAGACAGCTGAGCTGCAATACATACAGCAAGTTAGTGG

2400
LysLeuSerValThrValLeuSerGlnIleTyrProGlylleLysValArgGlnLeuCysLysLeuLeuArgGlyThrLysAlaLeuThrGluValIleProLeuThrGluGluAlaGluLeuGlu

AAAATTGAATTTGGGCAAGTCAGATTTACCGGAGATTAAGTAAGGCAATATGTAACTCTTAGAGGAACCAAGCACTAACAGAAGTAAATACCATAACAGAAGAGCAGCAGTGA

2500
LeuAlaGluAsnArgGluIleLeuLysGluProValHisGlyValTyrTyrAspProSerLysAspleuIleAlaGluIleGlnLysGlnGlyGlnGlyGlnTrpThrTyrGlnIleTyr

ACTGGGCAAAAAACAGAGATTTCAAAAGAACGAGTACATGAGCTGATTATGACCCATCAAAAGCACTTAATAGCAAAATACAGAAGCAGGGCAAGCCAAATGACATATCAAAITTA

2600
GlnGluProPheLysAsnLeuLysThrGlyLysTyrAlaArgThrArgGlyAlaHisThrAsnAspValLysGlnLeuThrGluAlaValGlnLysIleThrThrGluSerIleValIle

TCAAGAGCATTAAAAATCTGAAACAGGAAAAATATCCAAGAACGAGGGGTGCCCACTAATGATGTAACAATTAACAGAGCAGTGCAAAAATAACCAAGAAAGCATAGTAAT

2700
TrpGlyLysThrProLysPheLysLeuProIleGlnGlyGlnLeuThrTrpGlnTrpTrpGluThrTrpGluThrTrpGluThrTrpIleProLeuTrpGluTrpGluTrpGluTrp

ATGGGGAAGACTCTCAATTTAAATACCCTACCAAAAGAACATCGGAAACATCGGGAACAGTAGTGGCAAGCACTGAGTGGCAAGCACTGAGTCTGAGTGGGAGTTTGTCAATACCCCTCTCTT

2800
ValLysLeuTrpTyrGlnLeuGluLysGluProlleValGlyAlaGluThrPheTyrValAspGlyAlaAlaSerArgGluThrLysLeuGlyLysAlaGlyTyrValThrAsnArgGly

AGTGAAATTTAGTACCACTAGAGAAAGAACCACTAGTAGGAGCAGAAAGCTTCTATGTAGATGGGCACTAGCAGGAGACATAATAGGAAAGCAGCATATGTTACTAATAGAGG

2900
ArgGlnLysValValThrLeuThrAspThrThrAsnGlnLysThrGluLeuGlnAlaIleHis

LeuGlyIleIleGlnAlaGlnProAspLysSerGluSerGluLeuValAsnGlnIleIleGluGlnLeuLysLysGlyLysValTrpLeuAlaTrpValProAlaHisGlyGlyLeu
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3700
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3800
AlaSerAspPheAsnLeuProValValAlaLysGluIleValAlaSerCysAspLysCysGlnLeuLysGlyGluAlaMetHisGlyGlnValAspCysSerProGlyIleTrpGln
CGCTAGTGAATTTTAACTGCGCACCTGTAGTACGAAAGAAATAGTAGCCAGCTGTGATAAATGTGCGCTAAAGGAGCAAGCATGCGACAGTACAGTGTAGTCCGAGGAATATGGCA
3900
LeuAspCysThrHisLeuGluGlyLysValIleLeuValAlaValHisValAlaSerGlyTyrIleGluAlaGluValIleProAlaGluThrGlyGlnTrpAlaThrLeuLeu
ACTAGATTGTACACATTTTGAAGGAAAGTTATCTGCTGAGTACCTGATGACCACTGGATATATAGAAGCAAGATTATTCAGCAGCAAGGAGGAAACAGCATACTTTCTTT
4000
LysLeuAlaGlyArgTrpProValLysThrIleHisThrAspAsnGlySerAsnPheThrSerThrThrValLysAlaAlaCysTrpTrpAlaGlyIleLysGlnGluPheGlyIlePro
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4100
TrpAsnProGlnSerGlnGlyValGluSerMetAsnLysGluLeuLysLysIleIleGlyGlnValArgAspGlnAlaGluHisLeuLysThrAlaValGlnMetAlaValPheIle
CTCAATTCGCCAAGTCAAGGAGCTAGTAGAATCTATGAATAAAGAAATTAAGAAAAATATAGCCAGGTAGAGATCAGGCTGAACATCTTAACAGCAGCACTCAAAATGGCAGTATTCAT
4200
HisAsnPheLysArgLysGlyGlyIleGlyGlyTyrSerAlaGlyGluArgIleValAspIleIleAlaThrAspIleGlnThrLysGluLeuGlnLysGlnIleThrLysIleGlnAsn
CCACAATTTTAAAGAAAGGCGGATGGCGGCTACAGTGCACGGGAAAGAAATAGTAGACATAATAGCAACAGACATACAACTAAAGAAATACAAAAACAAATACAAAAATCAAAA
4300
PheArgValTyrTyrArgAspSerArgAspProLeuTrpLysGlyProAlaLysLeuLeuTrpLysGlyGluGlyAlaValValIleGlnAspAsnSerAspIleLysValProArg
ORF Q = CysGlnGlu
TTTTCGGCTTTATTACAGGACAGCAGAGATCCACTTTGGAAGGACAGCAAGCTCTCTGGAAGGTGAAGGGCAGTACTAATACAGATAATAGTACATAAACTAGTCCAAAG
4400
ArgLysAlaLysIleIleArgAspTyrGlyLysGlnMetAlaGlyAspAspCysValAlaSerArgGlnAspGluAsp
GluLysGlnArgSerLeuGlyIleGlnGluAsnArgTrpGlnValMetIleValTrpGlnValAspArgMetArgIleArgThrTrpLysSerLeuValLysHisMetTyrValSer
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4500
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4700
AspCysPheSerAspSerAlaIleArgLysAlaLeuLeuGlyHisIleValSerProArgCysGluTyrGlnAlaGlyHisAsnLysValGlySerLeuGluTyrLeuAlaLeuAlaAla
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5000
CATAATAAGAAATTCGCAACAACTGCTGTTATGCAATTTGAGAATGGGTGCGACATAGCAGAAATAGCGCTTACTCAACAGAGGAGAGCAAGAAATGGAGCAGTATCTAGACTAG
5100
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5200
GGAAGAGCGGAGACGCGACGAAGACCTCTCAAGGCACTGACAGTCACTCAAGTTTCTCTATCAAGAGTAGTAGTACTATGTAATGCAACCTATACAAATAGCAATAGCAGCATTAG
5300
5400
5500
5600
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5700
ValAlaMetArgValLysGluLysTyrGlnHisLeuTrpArgTrpGlyTrpLysTrpGlyThrMetLeuLeuGlyIleLeuMetIleCysSerAlaThrGluLysLeuTrpValThrVal
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5800
TyrTyrGlyValProValTrpLysGluAlaThrThrLeuPheCysAlaSerAspAlaLysAlaTyrAspThrGluValHisAsnValTrpAlaThrHisAlaCysValProTrpAsp
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5900
ProAsnProGlnGluValValLeuValAsnValTrpGluAsnMetTrpLysAsnAspMetAlaGlnMetHisGluAspIleIleSerLeuTrpAspGlnSerLeuLysPro
CCCAACCCCAAGAGTAGTATTGCTAATGTGACAAAAATTTTAACTGTGAAAAATGACATGTTAGAACAGATGATGAGGATATAATCAGTTTATGGATCAAAAGCTTAAAGCCA
6000
CysValLysLeuThrProLeuCysValSerLeuLysCysThrAspLeuGlyAsnAlaThrAsnThrAsnSerSerAsnThrAsnSerSerGlyGluMetMetMetGluLysGlyGlu
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6100
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6200
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AsnGlyThrGlyProCysThrAsnValSerThrValGlnCysThrHisGlyIleArgProValSerThrGlnLeuLeuAsnAlaGluGluValValIleArg
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SerAlaAsnPheThrAspAsnAlaThrIleIleValGlnLeuAsnGlnSerValGluIleAsnCysThrArgProAsnAsnAsnThrArgLysSerIleArgIleGlnArgGlyPro
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6500
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6600
GlyAsnAsnLysThrIleIlePheLysGlnSerSerGlyGlyAspProGluIleValThrHisSerPheAsnCysGlyGlyGluPhePheTyrCysAsnSerThrGlnLeuPheAsnSer
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6700
ThrTrpPheAsnSerThrTrpSerThrGluGlySerAsnAsnThrGluGlySerAspThrIleThrLeuProCysArgIleLysGlnPheIleAsnThrGlnGlnValGlnLysAla
ACTTGCTTAACTACTTGCAGTACTGAAGGCTCAATTAACACTGAAGGAGTGCACAACTACCACTCCCATGCGAAGATAAAACATTTTAAAGACTTGGCAGAGAGTAGGAAAGCA
6800
MetTyrGlnProIleSerGlnIleArgGlnThrHisSerAsnIleThrGlyLeuLeuThrArgAspGlyGlyAsnAsnAsnGlySerGluIlePheArgProGlyGlyGly
ATGTAATGCCCTCCCATCAGCGCAAAATAGATGTTTATCAAAATATTACAGGCTGCTATTAACAGAGATGCTGTAATAACACCAATGGTCCGAGTCTTCAGACCTGCGAGCAGCA
6900
AspMetArgAspAsnTrpArgSerGluLeuTyrLysTyrLysValValLysIleGluProLeuGlyValAlaProThrLysAlaLysArgArgValValGlnArgGluLysArgAlaVal
CATATCAGGCAATTTGAGCAAGTCAATATATAAATAAAGTAGTAAAAATGAACATTAGGAGTAGCAGCCACAGGCAAGAGAGAGCTGTCCACAGAGAAAAAGAGCAGCTG
7000
7100
7200

The nucleotide coordinates refer to the first base of the first triplet (1st triplet), of the first methionine (initiation) codon (Met) and of the stop codon (Stop). The numbers of amino acids and molecular weights are those calculated for unmodified precursor products starting at the first methionine through to the end, with the exception of pol. where the size and M_r refer to that of the whole orf.

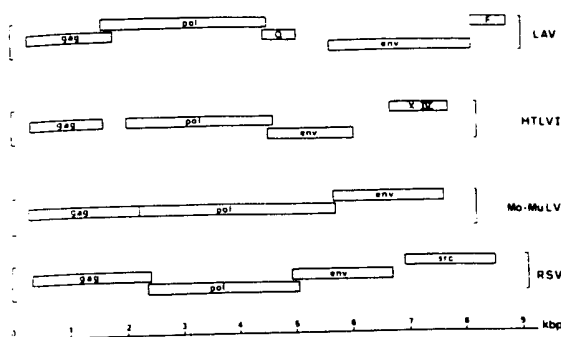


Figure 2. Comparison of the Genome Organization of LAV with Those of Human T Cell Leukemia/Lymphoma Virus Type I (HTLV-I) (Seiki et al., 1983), Moloney Murine Leukemia Virus (MoMuLV) (Shinnick et al., 1981), and Rous Sarcoma Virus (RSV) (Schwartz et al., 1983)

The positions and sizes of viral genes are drawn to scale (open boxes) and the viral genomes (RNA forms) are delimited by brackets.

sis of the primer, R+U5 was found to be 181 ± 1 bp (Figure 4). Thus R is 97 bp long and the cap site at its 5' end can be located. Finally, U3 is 456 bp long. The LAV LTR also contains characteristic regulatory elements: a polyadenylation signal sequence AATAAA 19 bp from the R-U5 junction, and the sequence ATATAAG, which is very likely the TATA box, 22 bp 5' of the cap site. There are no long direct repeats within the LTR. Interestingly, the LAV LTR shows some similarities to that of the mouse mammary tumor virus (MMTV) (Donehower et al., 1981). They both use tRNA^{lys} as a primer for (-) strand synthesis, whereas all other exogenous mammalian retroviruses known to date use tRNA^{pro} (Chen and Barker, 1984). They possess very similar polypurine tracts; that of LAV is AAAAGAAAAGGGGGG while that of MMTV is AAAAAAGAAAAAGGGGGG. It is probable that the viral (+) strand synthesis is discontinuous since the polypurine tract flanking the U3 element of the 3'LTR is found exactly duplicated in the 3' end of orf pol, at 4331-4346. In addition, MMTV and LAV are exceptional in that the U3 element can encode an orf. In the case of MMTV, U3 contains the whole orf while, in LAV, U3 contains 110 codons of the 3' half of orf F.

Viral Proteins

gag

Near the 5' extremity of the gag orf is a "typical" initiation codon (Kozak, 1984) (position 336), which is not only the first in the gag orf, but the first from the cap site. The precursor protein is 500 amino acids long. The calculated M_r of 55,841 agrees with the 55 kd gag precursor polypeptide (Luc Montagnier, unpublished results). The N-terminal amino acid sequence of the major core protein p25, obtained by microsequencing (Genetic Systems, personal communication), matches perfectly with the translated nucleotide sequence starting from position 732 (see Figure 1). This formally makes the link between the cloned LAV genome and the immunologically characterized LAV p25 protein. The protein encoded 5' of the p25 coding sequence is rather hydrophilic. Its calculated M_r of 14,866 is consistent with that of the gag protein p18. The 3' part of the gag region probably codes for the retroviral nucleic acid binding protein (NBP). Indeed, as in HTLV-I (Seiki et

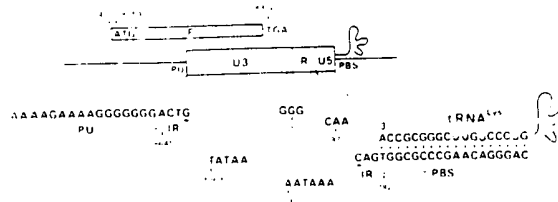


Figure 3. Schematic Representation of the LAV Long Terminal Repeat (LTR)

The LTR was reconstructed from the sequence of $\Delta J19$ by juxtaposing the sequences adjacent to the Hind III cloning sites. Sequencing of oligo(dT)-primed LAV DNA clone pLAV75 (Alizon et al., 1984) rules out the possibility of clustered Hind III sites in the R region of LAV. LTR are limited by an inverted repeat sequence (IR). Both of the viral elements flanking the LTR have been represented as tRNA primer binding site (PBS) for 5' LTR and polypurine track (PU) for 3' LTR. Also indicated are a putative TATA box, the cap site, polyadenylation signal (AATAAA), and polyadenylation site (CAA). The location of the open reading frame F (648 nucleotides) is shown above the LTR scheme.

al., 1983) and RSV (Schwartz et al., 1983), the motif Cys-X₂-Cys-X₂-Cys common to all NBP (Oroszlan et al., 1984) is found duplicated (nucleotides 1509 and 1572 in LAV sequence). Consistent with its function the putative NBP is extremely basic (17% Arg + Lys).

pol

The reverse transcriptase gene can encode a protein of up to 1003 amino acids (calculated M_r = 113,629). Since the first methionine codon is 92 triplets from the origin of the open reading frame, it is possible that the protein is translated from a spliced messenger RNA, giving a gag-pol polyprotein precursor.

The pol coding region is the only one in which significant homology has been found with other retroviral protein sequences, three domains of homology being apparent. The first is a very short region of 17 amino acids (starting at 1856). Homologous regions are located within the p15 gag^{RSV} protease (Dittmar and Moelling, 1978) and a polypeptide encoded by an open reading frame located between gag and pol of HTLV-I (Figure 5) (Schwartz et al., 1983; Seiki et al., 1983). This first domain could thus correspond to a conserved sequence in viral proteases. Its different locations within the three genomes may not be significant since retroviruses, by splicing or other mechanisms, express a gag-pol polyprotein precursor (Schwartz et al., 1983; Seiki et al., 1983). The second and most extensive region of homology (starting at 2048) probably represents the core sequence of the reverse transcriptase. Over a region of 250 amino acids, with only minimal insertions or deletions, LAV shows 38% amino acid identity with RSV, 25% with HTLV-I, and 21% with MoMuLV (Schinnick et al., 1981) while HTLV-I and RSV show 38% identity in the same region. A third homologous region is situated at the 3' end of the pol reading frame and corresponds to part of the pp32 peptide of RSV that has exonuclease activity (Misra et al., 1982). Once again, there is greater homology with the corresponding RSV sequence than with HTLV-I.

env

The env open reading frame has a possible initiator methionine codon very near the beginning (eighth triplet).

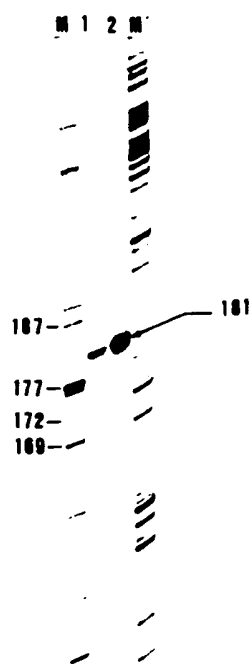


Figure 4. Synthesis of RNA-Primed LAV cDNA for R+U5 (Strong-Stop cDNA)

Lanes 1 and 2 show two different quantities of cDNA while lanes M and M' represent markers. The strong-stop cDNA is 181 bases long with a second, less intense band at 180. The error of estimation is ± 1 bp. This maps the major cap site to the second G residue of the sequence CTGGGTCT within the LTR, 24 nucleotides downstream of the TATA box. This guanosine residue is taken as the first base in the nucleotide sequence shown in Figure 1.

If so, the molecular weight of the presumed env precursor protein (861 amino acids, M_r calc = 97,376) is consistent with the known size of the LAV glycoprotein (110 kd and 90 kd after glycosidase treatment; Luc Montagnier, unpublished). There are 32 potential N-glycosylation sites (Asn-X-Ser/Thr), which are overlined in Figure 1. An interesting feature of env is the very high number of Trp residues at both ends of the protein. There are three hydrophobic regions, characteristic of the retroviral envelope proteins (Seiki et al., 1983), corresponding to a signal peptide (encoded by nucleotides 5815–5850 bp), a second region (7315–7350 bp), and a transmembrane segment (7831–7896 bp). The second hydrophobic region (7315–7350 bp) is preceded by a stretch rich in Arg + Lys. It is possible that this represents a site of proteolytic cleavage, which, by analogy with other retroviral proteins, would give an external envelope polypeptide and a membrane-associated protein (Seiki et al., 1983; Kiyokawa et al., 1984). A striking feature of the LAV envelope protein sequence is that the region following the transmembrane segment is of unusual length (150 residues). The env protein shows no

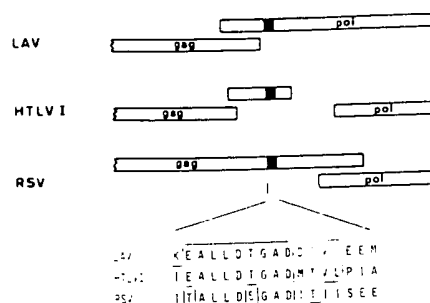


Figure 5. Location of a Short Stretch of Homology in the gag-pol Region of the LAV, HTLV-I (Seiki et al., 1983) and RSV (Schwartz et al., 1983) Genomes

Conserved amino acids are boxed. Homologous region is shown by the solid bar in the schema. Each virus is organized differently in this region but the sequence in the RSV genome maps to p15^{gag}, which has a protease-associated function.

homology to any sequence in protein data banks. The small amino acid motif common to the transmembrane proteins of all leukemogenic retroviruses (Cianciolo et al., 1984) is not present in LAV env.

Q and F

The location of orf Q is without precedent in the structure of retroviruses. Orf F is unique in that it is half-encoded by the U3 element of the LTR. Both orf have strong initiator codons (Kozak, 1984) near their 5' ends and can encode proteins of 192 amino acids (M_r calc = 22,487) and 206 amino acids (M_r calc = 23,316), respectively. Both putative proteins are hydrophilic (pQ 49% polar, 15.1% Arg + Lys; pF 46% polar, 11% Arg + Lys) and are therefore unlikely to be associated directly with membrane. The function for the putative proteins pQ and pF cannot be predicted, as no homology was found by screening protein sequence data banks. Between orf F and the pX protein of HTLV-I there is no detectable homology. Furthermore, their hydrophobicity/hydrophilicity profiles are completely different. It is known that retroviruses can transduce cellular genes—notably proto-oncogenes (Weinberg, 1982). We suggest that orfs Q and F represent exogenous genetic material and not some vestige of cellular DNA because LAV DNA does not hybridize to the human genome under stringent conditions (Alizon et al., 1984), and their codon usage is comparable to that of the gag, pol, and env genes (data not shown).

Relationship to Other Retroviruses

Although LAV is both morphologically and biochemically (Barré-Sinoussi et al., 1983) distinct to HTLV-I and -II, it remained possible that its genome was organized in a similar manner. The characteristic features of HTLV-I and -II genomes, which they share with the more distantly related bovine leukemia virus (BLV) (Rice et al., 1984), are not observed in the case of LAV. These are: a region 3' of the envelope gene consisting of a noncoding stretch (600–900 bp), followed by a coding sequence of 307–357 codons (X open reading frame), which may slightly overlap the U3 region of the LTR (Seiki et al., 1983; Rice et al., 1984; Sagata et al., 1984) and, second, the LTR being

Table 2. Comparison of the Size of the LAV LTR and LTR-Related Element to Those of Other Retroviruses

| | LTR | U3 | R | U5 | PU | PBS | IR |
|---------|-------|-------|-----|-----|-----|-----|----|
| LAV | 638 | 456 | 97 | 85 | 15 | LYS | 4 |
| HTLV-I | 759 | 355 | 228 | 176 | 12' | PRO | 4' |
| HTLV-II | 763 | 314 | 248 | 261 | 12' | PRO | 4' |
| MMTV | 1,332 | 1,197 | 11 | 124 | 19 | LYS | 8' |
| MoMuLV | 594 | 449 | 68 | 77 | 13 | PRO | 13 |
| RSV | 335 | 234 | 21 | 80 | 11 | TRP | 15 |
| SNV | 601 | 420 | 97 | 80 | 13 | PRO | 9 |

Adapted from Chen and Barker (1984).

i = imperfect match or tract.

SNV = spleen necrosis virus (Shimotohno and Temin, 1982).

composed of unusually long U5 and R elements and the polyadenylation signal being situated in U3 instead of R (Seiki et al., 1983; Sagata et al., 1984; Shimotohno et al., 1984). We show here that, in contrast, the 3' end of the LAV envelope gene overlaps an open reading frame, termed F, that has the coding capacity for 206 amino acids and extends within the LTR (110 amino acids are encoded by the U3 region). The putatively encoded polypeptide (pF), the primary structure of which can be deduced, does not show any homology with the theoretical X gene products of the HTLV/BLV family. Also, the U5 and R elements are shorter (Table 2) and the polyadenylation signal is located within R, as is the case for all retroviruses except the HTLV/BLV. Additionally, LAV uses tRNA^{lys} as (-) strand primer, as opposed to tRNA^{pro} employed by all other mammalian retroviruses except MMTV (Donehower et al., 1981). Those homologies detected between the polymerase and protease domains of LAV and HTLV are also found in several retroviruses, RSV in particular.

It has been reported that a cloned HTLV-III genome hybridizes ($T_m = 28^\circ\text{C}$) to sequences in the gag-pol and X regions of HTLV-I and -II; although restriction maps of cloned LAV and HTLV-III show almost perfect agreement (Hahn et al., 1984), we were unable to detect any such hybridization between LAV and HTLV-II ($T_m = 55^\circ\text{C}$) (Alizon et al., 1984). Indeed, there is a punctual region of homology between LAV and HTLV-I (23/27 nucleotides starting at position 1859 in the LAV sequence) but nothing significant between the two viruses in the X region of HTLV-I. One possible reason for this discrepancy is that HTLV-III is subtly different from LAV. However it was subsequently reported that there was very minimal, if any, homology between orf X (of HTLV-I) and HTLV-III (Shaw et al., 1984).

Discussion

Regulatory sequences carried by retroviral LTR are believed to be involved in specific interactions between the viral genome and the host cell (Srinivasan et al., 1984). The LTR sequences of LAV are unique among retroviruses. That could reflect an original mode of gene expression, possibly in relation to particular transcriptional factors present in the virus-harboring cell. This hypothesis can be tested by studying the regulatory activity of the LAV

LTR sequences in transient or long-term experiments involving an indicator gene and different cellular contexts.

The presence of the Q and F reading frames in addition to the conventional gag-pol-env set of genes is unexpected. One should now address the question of their role in the viral cycle and pathogenicity by trying to characterize their protein product(s). It is tempting to speculate on a role of such polypeptide(s) in T4 cells' mortality, a problem that can be studied by designing synthetic peptides for antibody production or by using site-directed mutagenesis of Q and F coding regions.

The peculiar genetic structure of LAV poses the question of its origin. The virus shares common tracts with other (apparently unrelated) retroviruses. For instance, the unusually large size of the outer membrane glycoprotein (env) and a comparably sized genome are also observed in the case of lentiviruses such as Visna (Harris et al., 1981; Querat et al., 1984). The presence of a large part of the F open reading frame in the LTR, and the use of tRNA^{lys} as a primer for (-) strand synthesis, is reminiscent of the mouse mammary tumor virus. On the other hand, homologies in the pol gene would suggest that the LAV is closer to RSV than to any other retroviruses. Obviously, no clear picture can be drawn from the DNA sequence analysis as far as phylogeny is concerned. Thus, it may well be that LAV defines a new group of retroviruses that have been independently evolving for a considerable period of time, and not simply a variant recently derived from a characterized viral family. Both epidemiology and pathogeny of AIDS should be reconsidered with this idea in mind, when trying to answer such questions as these: Are there other human or animal diseases that are associated with similarly organized viruses? Is there a precursor to AIDS-associated virus(es) normally present, in latent form, in human populations? What triggered in this case the recent spreading of pathogenic derivatives?

Experimental Procedures

M13 Cloning and Sequencing

Total λ 19 DNA was sonicated, treated with the Klenow fragment of DNA polymerase plus deoxyribonucleotides (2 hr, 16°C), and fractionated by agarose gel electrophoresis. Fragments of 300–600 bp were excised, electroeluted, and purified by Elutip (Schleicher and Schüll) chromatography. DNA was ethanol-precipitated using 10 μg dextran T40 (Pharmacia) as carrier and ligated to dephosphorylated, Sma I-cleaved M13mp8 RF DNA using T4 DNA and RNA ligases (16 hr, 16°C) and transfected into *E. coli* strain TG-I. Recombinant clones were detected by plaque hybridization using the appropriate ^{32}P -labeled LAV restriction fragments as probes. Single-stranded templates were prepared from plaques exhibiting positive hybridization signals and were sequenced by the dideoxy chain termination procedure (Sanger et al., 1977) using α - ^{32}S -dATP (Amersham, 400 Ci/mmol) and buffer gradient gels (Biggen et al., 1983). Sequences were compiled and analyzed using the programs of Staden adapted by B. Caudron for the Institut Pasteur Computer Center (Staden, 1982).

Strong-Stop cDNA

LAV virions from infected T lymphocyte (Barré-Sinoussi et al., 1983) culture supernatant were pelleted through a 20% sucrose cushion and the cDNA (-) strand was synthesized as described previously (Alizon et al., 1984) except that no exogenous primer was used. After alkaline hydrolysis (0.3 M NaOH, 30 min, 65°C), neutralization, and phenol extraction, the cDNA was ethanol-precipitated and loaded onto a 6%

acrylamide/8 M urea sequencing gel with sequence ladders as size markers.

Acknowledgments

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Isolation of a T-Lymphotropic Retrovirus from a Patient at Risk for Acquired Immune Deficiency Syndrome (AIDS)

Abstract. A retrovirus belonging to the family of recently discovered human T-cell leukemia viruses (HTLV), but clearly distinct from each previous isolate, has been isolated from a Caucasian patient with signs and symptoms that often precede the acquired immune deficiency syndrome (AIDS). This virus is a typical type-C RNA tumor virus, buds from the cell membrane, prefers magnesium for reverse transcriptase activity, and has an internal antigen (p25) similar to HTLV p24. Antibodies from serum of this patient react with proteins from viruses of the HTLV-I subgroup, but type-specific antisera to HTLV-I do not precipitate proteins of the new isolate. The virus from this patient has been transmitted into cord blood lymphocytes, and the virus produced by these cells is similar to the original isolate. From these studies it is concluded that this virus as well as the previous HTLV isolates belong to a general family of T-lymphotropic retroviruses that are horizontally transmitted in humans and may be involved in several pathological syndromes, including AIDS.

The acquired immune deficiency syndrome (AIDS) has recently been recognized in several countries (1). The disease has been reported mainly in homosexual males with multiple partners, and epidemiological studies suggest horizontal transmission by sexual routes (2) as well as by intravenous drug administration (3), and blood transfusion (4). The pronounced depression of cellular immunity that occurs in patients with AIDS and the quantitative modifications of subpopulations of their T lymphocytes (5) suggest that T cells or a subset of T cells might be a preferential target for the putative infectious agent. Alternatively, these modifications may result from subsequent infections. The depressed cellular immunity may result in serious opportunistic infections in AIDS patients, many of whom develop Kaposi's sarcoma (1). However, a picture of persistent multiple lymphadenopathies has also been described in homosexual males (6) and infants (7) who may or may not develop AIDS (8). The histological aspect of such lymph nodes is that of reactive hyperplasia. Such cases may correspond to an early or a milder form of the disease. We report here the isolation of a novel retrovirus from a lymph node of a homosexual patient with multiple lymphadenopathies. The virus appears to be a member of the human T-cell leukemia virus (HTLV) family (9).

The retrovirus was propagated in cultures of T lymphocytes from a healthy adult donor and from umbilical cord blood of newborn humans. Viral core proteins were not immunologically related to the p24 and p19 proteins of subgroup I of HTLV (9). However, serum of the patient reacted strongly with surface antigen (or antigens) present on HTLV-I-infected cells. Moreover, the ionic requirements of the viral reverse transcriptase were close to that of HTLV. Re-

cently, a type-C retrovirus was also identified in T cells from a patient with hairy cell leukemia. Analysis of the proteins of this virus showed they were related to, but clearly different from, proteins of previous HTLV isolates (10). Moreover, recent studies of the nucleic acid sequences of this new virus show it is less than 10 percent homologous to the earlier HTLV isolates (11). This virus was called HTLV-II to distinguish it from all the earlier, highly related viruses termed

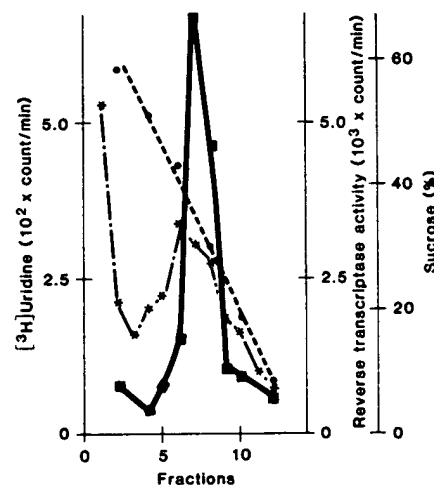


Fig. 1. Analysis of virus from patient 1 on sucrose gradients. Cord blood T lymphocytes infected with virus from patient 1 were labeled for 18 hours with [³H]uridine (28 Ci/mmole, Amersham; 20 μ Ci/ml). Cell-free supernatant was ultracentrifuged for 1 hour at 50,000 rev/min. The pellet was resuspended in 200 μ l of NTE buffer (10 mM tris, pH 7.4, 100 mM NaCl, and 1 mM EDTA) and was centrifuged over a 3-ml linear sucrose gradient (10 to 60 percent) at 55,000 rev/min for 90 minutes in an IEC type SB 498 rotor. Fractions (200 μ l) were collected, and 30 μ l samples of each fraction were assayed for DNA polymerase activity with 5 mM Mg²⁺ and poly(A) · oligo(dT)₁₂₋₁₈ as template primer; a 20- μ l portion of each fraction was precipitated with 10 percent trichloroacetic acid and then filtered on a 0.45- μ m Millipore filter. The ³H-labeled acid precipitable material was measured in a Packard β counter.

HTLV-I. The new retrovirus reported here appears to also differ from HTLV-II. We tentatively conclude that this virus, as well as all previous HTLV isolates, belong to a family of T-lymphotropic retroviruses that are horizontally transmitted in humans and may be involved in several pathological syndromes, including AIDS.

The patient was a 33-year-old homosexual male who sought medical consultation in December 1982 for cervical lymphadenopathy and asthenia (patient 1). Examination showed axillary and inguinal lymphadenopathies. Neither fever nor recent loss of weight were noted. The patient had a history of several episodes of gonorrhea and had been treated for syphilis in September 1982. During interviews he indicated that he had had more than 50 sexual partners per year and had traveled to many countries, including North Africa, Greece, and India. His last trip to New York was in 1979.

Laboratory tests indicated positive serology (immunoglobulin G) for cytomegalovirus (CMV) and Epstein-Barr virus. Herpes simplex virus was detected in cells from his throat that were cultured on human and monkey cells. A biopsy of a cervical lymph node was performed. One sample served for histological examination, which revealed follicular hyperplasia without change of the general architecture of the lymph node. Immunohistological studies revealed, in paracortical areas, numerous T lymphocytes (OKT3⁺). Typing of the whole cellular suspension indicated that 62 percent of the cells were T lymphocytes (OKT3⁺), 44 percent were T-helper cells (OKT4⁺), and 16 percent were suppressor cells (OKT8⁺).

Cells of the same biopsied lymph node were put in culture medium with phytohemagglutinin (PHA), T-cell growth factor (TCGF), and antiserum to human α interferon (12). The reason for using this antiserum was to neutralize endogenous interferon which is secreted by cells chronically infected by viruses, including retroviruses. In the mouse system, we had previously shown that antiserum to interferon could increase retrovirus production by a factor of 10 to 50 (13). After 3 days, the culture was continued in the same medium without PHA. Samples were regularly taken for assay of reverse transcriptase and for examination in the electron microscope.

After 15 days of culture, a reverse transcriptase activity was detected in the culture supernatant by using the ionic conditions described for HTLV-I (14). Virus production continued for 15 days

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and decreased thereafter, in parallel with the decline of lymphocyte proliferation. Peripheral blood lymphocytes cultured in the same way were consistently negative for reverse transcriptase activity, even after 6 weeks. Cytomegalovirus could be detected, upon prolonged cocultivation with MRC5 cells, in the original biopsy tissue, but not in the cultured T lymphocytes at any time of the culture.

Virus transmission was attempted with the use of a culture of T lymphocytes established from an adult healthy donor of the Blood Transfusion Center at the Pasteur Institute. On day 3, half of the culture was cocultivated with lymphocytes from the biopsy after centrifugation of the mixed cell suspensions. Reverse transcriptase activity could be detected in the supernatant on day 15 of the coculture but was not detectable on days 5 and 10. The reverse transcriptase had the same characteristics as that released by the patient's cells and the amount released remained stable for 15 to 20 days. Cells of the uninfected culture of the donor lymphocytes did not release reverse transcriptase activity during this period or up to 6 weeks when the culture was discontinued.

The cell-free supernatant of the infected coculture was used to infect 3-day-old cultures of T lymphocytes from two umbilical cords, LC1 and LC5, in the presence of Polybrene (2 µg/ml). After a lag period of 7 days, a relatively high titer of reverse transcriptase activity was detected in both of the cord lymphocyte cultures. Identical cultures, which had not been infected, remained negative. These two successive infections clearly show that the virus could be propagated on normal lymphocytes from either newborns or adults.

That this new isolate was a retrovirus was further indicated by its density in a sucrose gradient, which was 1.16, and by its labeling with [³H]uridine (Fig. 1). Electron microscopy of the infected umbilical cord lymphocytes showed characteristic immature particles with dense crescent (C-type) budding at the plasma membrane (Fig. 2).

Virus-infected cells from the original biopsy as well as infected lymphocytes from the first and second viral passages were used to determine the optimal requirements for reverse transcriptase activity and the template specificity of the enzyme. The results were the same in all instances. The reverse transcriptase activity displayed a strong affinity for poly(adenylate · oligodeoxythymidylate) [poly(A) · oligo(dT)], and required Mg²⁺ with an optimal concentration (5 mM) slightly lower than that for HTLV

(14) and an optimal pH of 7.8. The reaction was not inhibited by actinomycin D. This character, as well as the preferential specificity for riboseadenylate · deoxythymidylate over deoxyadenylate · deoxythymidylate, distinguish the viral enzyme from DNA-dependent polymerases.

We then determined whether or not this isolate was indistinguishable from HTLV-I isolates. Human T-cell leukemia virus has been isolated from cultured T lymphocytes of patients with T lymphomas and T leukemias [for a review, see (9)]. The antibodies used were specific for the p19 and p24 core proteins of

HTLV-I. A monoclonal antibody to p19 (15) and a polyclonal goat antibody to p24 (16) were used in an indirect fluorescence assay against infected cells from the biopsy of patient 1 and lymphocytes obtained from a healthy donor and infected with the same virus. As shown in Table 1, the virus-producing cells did not react with either type of antibody, whereas two lines of cord lymphocytes chronically infected with HTLV (17) and used as controls showed strong surface fluorescence.

When serum from patient 1 was tested against infected lymphocytes from the biopsy the surface fluorescence was as

Table 1. Indirect immunofluorescence assay. Cells were washed with phosphate-buffered saline (PBS) and resuspended in the same buffer. Portions (5×10^4 cells) were spotted on slides, air-dried and fixed for 10 minutes at room temperature in acetone. Slides were stored at -80°C until use. Twenty microliters of either monoclonal antibody to HTLV p19 (diluted 1/400 in PBS) or goat antibody to HTLV p24 (diluted 1/400 in PBS) or serum from patient 1 diluted 1/10 in PBS was applied to cells and incubated for 45 minutes at 37°C . The appropriate fluorescein-conjugated antiserum (antiserum to mouse, goat, or human immunoglobulin G) was diluted and applied to the fixed cells for 30 minutes at room temperature. Slides were then washed three times in PBS. Cells were stained with Evans blue solution for 15 minutes and then washed extensively with water before microscopic examination.

| Cell type | Immunofluorescence (percent positive) | | |
|----------------------------------|---------------------------------------|-----------------|----------------------|
| | Antibody to p19 | Antibody to p24 | Serum from patient 1 |
| Normal blood lymphocytes | | | |
| N 10916 | — | — | — |
| LC ₁ | — | — | — |
| HTLV-producing cells | | | |
| C ₉ /PL | + (90 to 100) | + (90 to 100) | + (90 to 100) |
| C ₁₀ /MJ ₂ | + (90 to 100) | + (90 to 100) | + (90 to 100) |
| Virus-producing cells from | | | |
| Patient 1 | — | — | + (90 to 100) |
| LC ₁ /patient 1 | — | — | ± (0.5 to 2) |
| Patient 2 | — | — | + (90 to 100) |

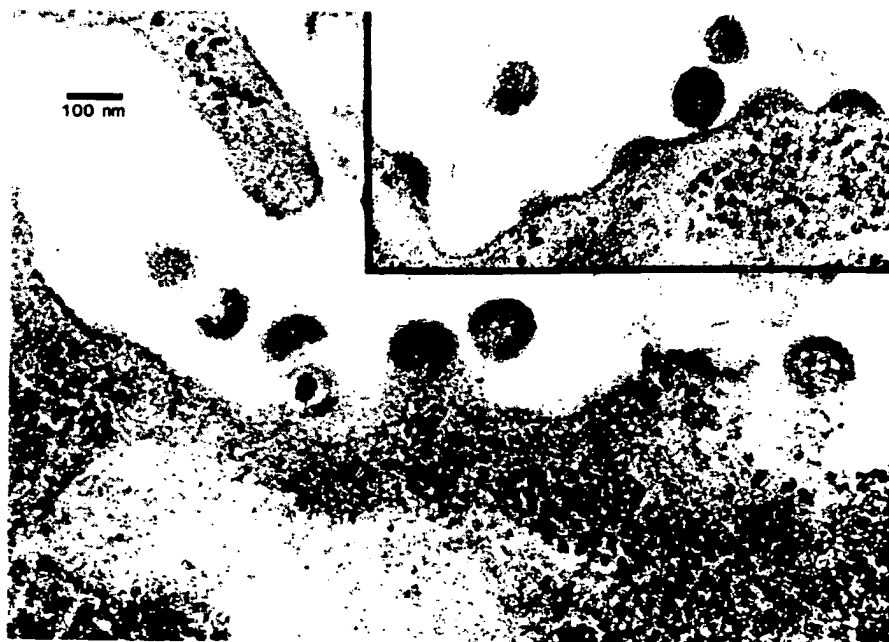


Fig. 2. Electron microscopy of thin sections of virus-producing cord lymphocytes. The inset shows various stages of particle budding at the cell surface.

intense as that of the control HTLV-producing lines. This suggests that serum of the patient contains antibodies that recognize a common antigen present on HTLV-I-producing cells and on the patient's lymphocytes. Similarly, cord lymphocytes infected with the virus from patient 1 did not react with antibodies to p19 or p24. Only a minor proportion of the cells (about 1 percent) reacted with the patient's serum. This may indicate that only this fraction of the cells was infected and produced virus. Alternatively, the antigen recognized by the patient's serum may contain cellular determinants that show less expression in T lymphocytes of newborns.

We also cultured T lymphocytes from a lymph node of another patient (patient 2) who presented with multiple adenopathies and had been in close contact with an AIDS case. These lymphocytes did not produce viral reverse transcriptase; however, they reacted in the immunofluorescence assay with serum from patient 1. Moreover, serum from patient 2 react-

ed strongly with control HTLV-producing lines (not shown). In order to determine which viral antigen was recognized by antibodies present in the two patients' sera, several immunoprecipitation experiments were carried out. Cord lymphocytes infected with virus from patient 1 and uninfected controls were labeled with [³⁵S]methionine for 20 hours. Cells were lysed with detergents, and a cytoplasmic S10 extract was made. Labeled virus released in the supernatant was banded in a sucrose gradient. Both materials were immunoprecipitated by antiserum to HTLV-I p24, by serum from patients 1 and 2, and by serum samples from healthy donors. Immunocomplexes were analyzed by polyacrylamide gel electrophoresis under denaturing conditions. Figure 3 shows that a p25 protein present in the virus-infected cells from patient 1 and in LC1 cells infected with this virus, was specifically recognized by serum from patients 1 and 2 but not by antiserum to HTLV-I p24 or serum of normal donors. Conversely, the p24

present in control HTLV-infected cell extracts was recognized by antibodies to HTLV but not by serum from patient 1. A weak band (lane 2, Fig. 3B) could hardly be seen with serum from patient 2, suggesting some similarities of the p25 protein from this patient's cells with HTLV-I p24. When purified, labeled virus from patient 1 was analyzed under similar conditions, three major proteins could be seen: the p25 protein and proteins with molecular weights of 80,000 and 45,000. The 45K protein may be due to contamination of the virus by cellular actin which was present in immunoprecipitates of all the cell extracts (Fig. 3).

These results, together with the immunofluorescence data, indicate that the retrovirus from patient 1 contains a major p25 protein, similar in size to that of HTLV-I but different immunologically. The DNA sequences of these and other members of the HTLV family are being compared. All attempts to infect other cells such as a B-lymphoblastoid cell line (Raji), immature or pre-T cell lines (CEM, HSB₂), and normal fibroblasts (feline and mink lung cell lines) were unsuccessful.

The role of this virus in the etiology of AIDS remains to be determined. Patient 1 had circulating antibodies against the virus, and some of the latter persisted in lymphocytes of his lymph node (or nodes). The virus-producing lymphocytes seemed to have no increased growth potential in vitro compared to the uninfected cells. Therefore, the multiple lymphadenopathies may represent a host reaction against the persistent viral infection rather than hyperproliferation of virus-infected lymphocytes. Other factors, such as repeated infection by the same virus or other bacterial and viral agents may, in some patients, overload this early defense mechanism and bring about an irreversible depletion of T cells involved in cellular immunity.

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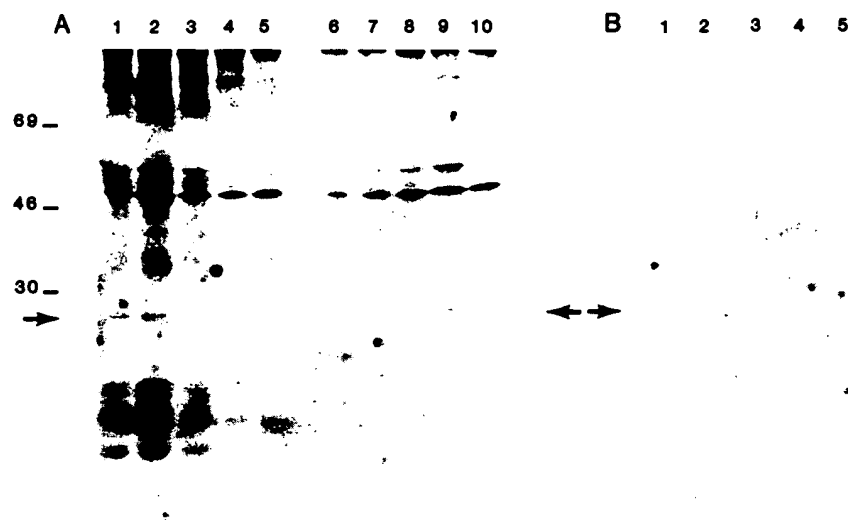


Fig. 3. Immunoprecipitation of ³⁵S-labeled viral proteins. Cord blood T-lymphocytes infected with virus from patient 1 were incubated overnight in culture medium containing one-fifth of the normal concentrations of methionine in minimum essential medium, [³⁵S]methionine (1500 Ci/mmole, Amersham; 50 μ Ci/ml), and 10 percent dialyzed fetal calf serum. The virus was purified by banding on a sucrose gradient as described in Fig. 1. Labeled cells were resuspended in 10 μ l of saline and then lysed with 90 μ l of RIPA buffer (18) containing aprotinin (500 U/ml; Zymofren, Specia) at 4°C for 15 minutes. The supernatant of a 10,000g centrifugation of the cell extract was used for immunoprecipitation. A similar extract was made from HTLV-producing C₉₁/PL cells (17). (A) Portions (20 μ l) of cell extracts were mixed with 6 μ l of serum, incubated for 2 hours at 37°C and overnight at +4°C. Then, 60 μ l of a suspension of Protein A-Sepharose (10 mg/ml in RIPA buffer) were added. After 45 minutes of incubation at 4°C, immunocomplexes bound to Protein A-Sepharose were washed five times with RIPA buffer by centrifugation, heated for 3 minutes at 100°C in denaturing buffer and electrophoresed on 12.5 percent polyacrylamide-SDS slab gel (19). Lanes 1 to 5: Extract of LC₁ cells infected with virus from patient 1 and tested against 1, serum from patient 1; 2, serum from patient 2; 3, serum of a healthy donor; 4, goat antiserum to HTLV-Ip24; 5, normal goat serum. Lanes 6 to 10: C₉₁/PL (HTLV-producing) cell extract tested with: 6, serum from patient 1; 7, serum from patient 2; 8, serum of a healthy donor; 4, goat antiserum to HTLV-Ip24; 5, normal goat serum. (B) Portions (20 μ l) of the band containing virus from patient 1 were treated with various antisera and processed as described for cell extracts. Lane 1, serum from patient 1; 2, serum from patient 2; 3, serum of a healthy donor; 4, serum of another healthy donor; 5, goat antiserum to HTLV-Ip24. Arrows indicate the p24-p25 protein. Molecular weights (in thousands) are indicated on the left.

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- We thank Dr. Fradellizi for gifts of T-cell growth factor, R. C. Gallo for providing antibodies to HTLV and for HTLV-producing cells, Mrs. Le François for preparation of the cord lymphocytes, M. Laverne (Institut Pasteur, Production) for gifts of fluorescein-conjugated antisera, F. Huraud for performing some of the HTLV tests, and members of the French Working Group on AIDS for helpful discussion.

19 April 1983

Heinz-Body Hemolytic Anemia from the Ingestion of Crude Oil: A Primary Toxic Effect in Marine Birds

Abstract. Hemolytic anemia developed in young herring gulls and Atlantic puffins given daily oral doses of a Prudhoe Bay crude oil. Anemia developed 4 to 5 days after the initiation of oil ingestion and was accompanied by Heinz-body formation and a strong regenerative response. The data evince a toxic effect on circulating red blood cells involving an oxidative biochemical mechanism and the first clear evidence of a primary mechanism of toxicity from the ingestion of crude oil by birds.

Petroleum oils regularly enter the marine environment through spills, runoff, and seepage (1). Large numbers of birds have died in association with marine oil spills (2), and the effects of oil on birds have been studied experimentally. Birds that become oiled ingest oil while preening (3), and oral doses of several petroleum oils have produced a wide range of sublethal toxic changes affecting growth, reproduction, osmoregulation, steroid metabolism, and hepatic function (4-6). Thus there is a firm basis for concern that oil pollution may produce subtle, sublethal effects in wild birds that impair reproduction or survival. This concern is heightened by the current emphasis on offshore oil development.

We report here that young herring gulls (*Larus argentatus*) and Atlantic puffins (*Fratercula arctica*) developed a severe hemolytic anemia after several days of oral dosing with a Prudhoe Bay crude oil (PBCO). Our data indicate that this was a primary toxic effect in which oxidative chemical processes damaged red blood cells in the peripheral circulation, and they constitute the first clear evidence of a primary toxic mechanism in experimental studies of the toxicity of ingested crude oil in birds.

In our initial experiments we used herring gull nestlings 2 to 3 weeks old,

taken from a coastal colony. These young birds adjust well to captivity and tolerate the manipulation required in laboratory work. Gulls were collected on Great Island, 50 km south of St. John's, Newfoundland, and held in pens at Memorial University of Newfoundland, St. John's (7). Pens were partially bedded with hay, and the birds were fed unlimited amounts of capelin (*Mallotus villosus*) and seawater. When all the birds were

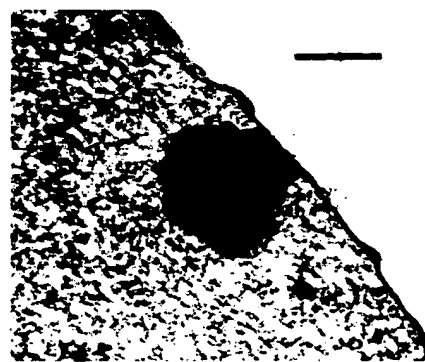


Fig. 1. Transmission electron micrograph of a Heinz body attached to the plasma membrane of a red blood cell from a herring gull which ingested 20 ml of Prudhoe Bay crude oil per kilogram per day for 4 days (experiment 1). This cell is a ghost erythrocyte, with most of the free hemoglobin lost from its cytoplasm; bar, 200×10^{-6} mm.

gaining weight (3 to 5 days after capture), they were divided into experimental groups of similar mean body weight (460 g) and weight distribution. Blood (1 ml) was taken from each bird, and experiments were begun immediately. In experiment 1, the birds were given either 10 or 20 ml of PBCO (8) per kilogram of body weight per day in gelatin capsules; controls received empty capsules. Dosing continued daily. Blood samples were drawn into heparinized tubes on day 5, and routine hematological measurements were made (9). Values for all groups prior to dosing and for controls on day 5 of dosing did not differ significantly. On day 5, blood taken from oil-dosed birds was dark brown and failed to redden on mixing with air; this result suggested a marked reduction in oxygen-carrying hemoglobin. The birds receiving oil were severely anemic, with packed cell volumes (PCV) reduced by 43 and 50 percent (Table 1). The plasma from these birds was rusty red, an indication of hemolysis either intravascularly or during sample handling. A strong regenerative response to the anemia was evident in the high reticulocyte count (Table 1). After the birds were killed, there was no evidence of trauma, enteric bleeding, or other hemorrhage. These data are sufficient to permit the classification of this anemia arising from oil ingestion as a hemolytic anemia (10).

Heinz bodies were abundant in erythrocytes from oil-dosed birds (Table 1). These were identified by two different staining techniques applied to fresh samples and in sections studied by light and transmission electron microscopy (Fig. 1) (9). Heinz bodies are dense granular masses in red cells thought to consist of precipitates of hemoglobin oxidized in the protein moiety. They are a classical feature of toxic hemolytic anemias produced by a variety of dissimilar chemicals linked mechanistically by the ability to cause destructive oxidative reactions in red cells (11). The presence of Heinz bodies is good evidence of a primary toxicosis of red cells and of an oxidative biochemical mechanism of toxicity. To probe other possible sites of destructive oxidative processes in these red cells, we measured methemoglobin, sulfhemoglobin, and reduced glutathione (GSH) in whole blood and extractable fluorescence (EF) in red cell membranes as an indicator of membrane lipid peroxidation (12). No sulfhemoglobin was detected. The range of methemoglobin values was wide in all groups, and experimental animals did not differ significantly from controls. Means and standard deviations for the percentages of methemoglobin

Nucleic acid hybridisation

a practical approach

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Cover illustration. On the left is an autoradiograph of λ gt11 recombinants containing *Dicystostelium discoideum* genomic DNA inserts, screened on nitrocellulose filters with a nick-translated 4.1 kb repetitive genomic fragment by the method of Benton and Davis (see Chapter 5). On the right is an autoradiograph of part of *Drosophila melanogaster* polytene chromosome 2R (stained with Giemsa) showing multiple sites of *in situ* hybridisation by the mobile element pDm1 137 (Dawid *et al.* (1981) Cell 25, 399); magnification 1300 x. The photographs were kindly supplied by Ms. P. Jagger and Mr. D.P. Ramji (Department of Biochemistry, University of Leeds, UK) and Dr. M.L. Pardue (M.I.T., Cambridge, USA), respectively.

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- (vii) Hybridise the DNA or RNA probe to the coupled cellulose for at least 18 h in buffer B or C, respectively, at 65°C using 1 ml microcentrifuge tubes mounted on a rotating shaker inside a 65°C oven. Keep the reaction volumes to a minimum (300 μ l/100 mg cellulose).
- (viii) Wash the cellulose nine times with buffer B for DNA probes or buffer C for RNA probes, to remove unhybridised nucleic acids which will be enriched for the desired probe sequences.
- (ix) Remove unwanted, hybridised sequences from the cellulose by washing nine times with buffer A. This regenerates the cellulose. The enriched sequences from step (viii) can be re-applied to the cellulose several times to achieve the desired degree of enrichment.

4. LABELLING NUCLEIC ACIDS FOR USE AS PROBES

4.1 Radioactive Labelling Methods

4.1.1 Choice of Radioisotope

The following radioactive isotopes are those most commonly used in nucleic acid hybridisations:

| Isotope | Half-life | Type of decay | Energy |
|------------------|-------------|---------------|--------|
| ^{32}P | 14.3 days | β | high |
| ^{125}I | 60.0 days | γ | medium |
| ^3H | 12.35 years | β | low |

For nucleic acid hybridisation in solution and on filters, ^{32}P is the isotope of choice since its high energy results in short scintillation counting times and short autoradiographic exposures. Each of the four deoxyribonucleotides is available in an α - ^{32}P -labelled form, of high or low specific radioactivity, suitable for incorporation into DNA using one of the polymerase reactions (Sections 4.1.2, 4.1.3). In addition, [γ - ^{32}P]ATP is also available for 5' end-labelling RNA or DNA using polynucleotide kinase (Section 4.1.4). Similarly, [α - ^{32}P]cordycepin triphosphate can be used for 3' end-labelling (Section 4.1.5).

The traditional isotope of choice for *in situ* hybridisation (Chapter 8) was ^3H since its low energy results in low backgrounds. However, a serious consequence of this low energy is very long time periods for autoradiographic exposure of slides. Therefore, ^{125}I is now often used instead. Nucleotides labelled with either isotope are commercially available for use in polymerase reactions.

4.1.2 Nick Translation

The process of 'nick translation' utilises DNase I to create single-strand nicks in double-stranded DNA. The 5' \rightarrow 3' exonuclease and 5' \rightarrow 3' polymerase actions of *E. coli* DNA polymerase I are then used to remove stretches of single-stranded DNA starting at the nicks and replace them with new strands made by the incorporation of labelled deoxyribonucleotides (30.31). As a result, each nick moves along the DNA strand being repaired in a 5' \rightarrow 3' direction ('nick translation'). This technique is shown diagrammatically in Figure 2.

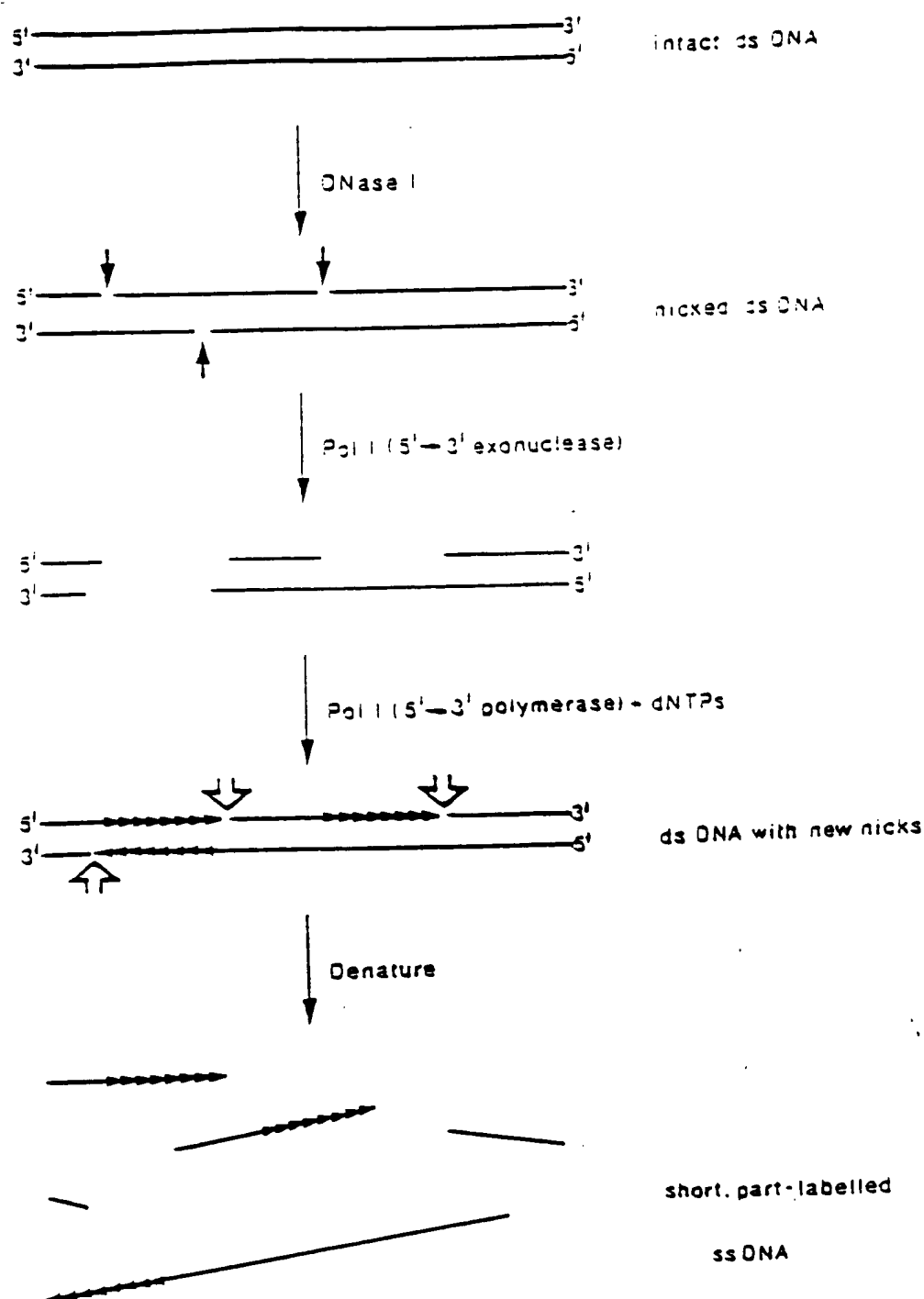


Figure 2. The preparation of probes by nick translation. \uparrow , original nick position; ∇ , final nick position; \bullet , labelled strand; Pol I, *E. coli* DNA polymerase I; dNTPs, deoxyribonucleoside triphosphates; ds and ss DNA, double- and single-stranded DNA, respectively.

Nick translation can utilise any deoxyribonucleotide labelled with ^{32}P in the α position. [^{125}I]-, [^3H]- and non-radioactive biotinylated nucleotides can also be incorporated. With α - ^{32}P -labelled nucleotides, final specific activities of 5×10^6 d.p.m./ μg DNA can be achieved. The detailed protocol is as follows.

Preparation of Nucleic Acid Probes

- (i) To a microcentrifuge tube, add:

0.5–1 μg DNA
2 μl 0.05 mM dATP
2 μl 0.05 mM dGTP
2 μl 0.05 mM dTTP
3.5 μl [α - ^{32}P]dCTP (400–2000 Ci/mmol; 10 mCi/ml)
5 μl 10 x nick-mix (50 mM MgCl_2 , 500 $\mu\text{g/ml}$ BSA, 0.5 M Tris-HCl, pH 7.5)
1 μl 3% 2-mercaptoethanol
water to a final volume of 48 μl

For higher specific activity probes, the unlabelled dTTP can be replaced with 3.5 μl [α - ^{32}P]dTTP (400–2000 Ci/mmol; 10 mCi/ml).

- (ii) Mix well; add 1 μl of DNase I (2 ng/ml) freshly diluted from a 2 mg/ml stock (stored at -20°C).
- (iii) Add 1 μl of DNA polymerase I (5 units). Incubate for 2 h at 16°C .
- (iv) Stop the reaction by adding 100 μl stop-mix (12.5 mM EDTA, 0.5% SDS, 10 mM Tris-HCl, pH 7.5).
- (v) Chromatograph the mixture on a small Sephadex G-50 column (e.g., in a siliconised Pasteur pipette) equilibrated in 3 x SSC. The DNA is excluded from the matrix and elutes ahead of the unincorporated deoxyribonucleotides. Collect 3-drop fractions from the column into microcentrifuge tubes and count directly by Cerenkov counting.
- (vi) Pool the fractions containing DNA.

The advantages of using nick translation as a labelling method are:

- (a) the simplicity of the reaction;
- (b) the uniform labelling of the DNA;
- (c) the high specific activities achieved.

The disadvantage is the nicking itself, which results in short single-stranded probe molecules in the hybridisation reaction.

An alternative protocol for nick translation is given in Chapter 8, Table 3.

4.1.3. Labelling with T4 DNA Polymerase

T4 DNA polymerase possesses two activities: a 3'–5' exonuclease activity and a 5'–3' polymerase (32,33). In the absence of exogenous deoxyribonucleoside triphosphates, only the exonuclease is active. It is more than 200 times as active as the exonuclease of DNA polymerase I and, under normal conditions, can remove 25 nucleotides per minute. When nucleotides are added, the polymerase activity predominates. The T4 DNA polymerase reaction is shown diagrammatically in Figure 3.

T4 polymerase will utilise any deoxyribonucleoside triphosphate labelled with ^{32}P in the α position or with ^3H or ^{125}I but has not yet been tested with biotinylated nucleotides. The detailed protocol is given below. Note that if the DNA to be labelled is a circular recombinant plasmid DNA, it must be first linearised by cutting with a suitable restriction enzyme.

- (i) At 0°C , dilute an aliquot of T4 DNA polymerase to 0.4 O'Farrell units/ μl (33) using the storage buffer recommended by the manufacturer as the diluent. Mix well.

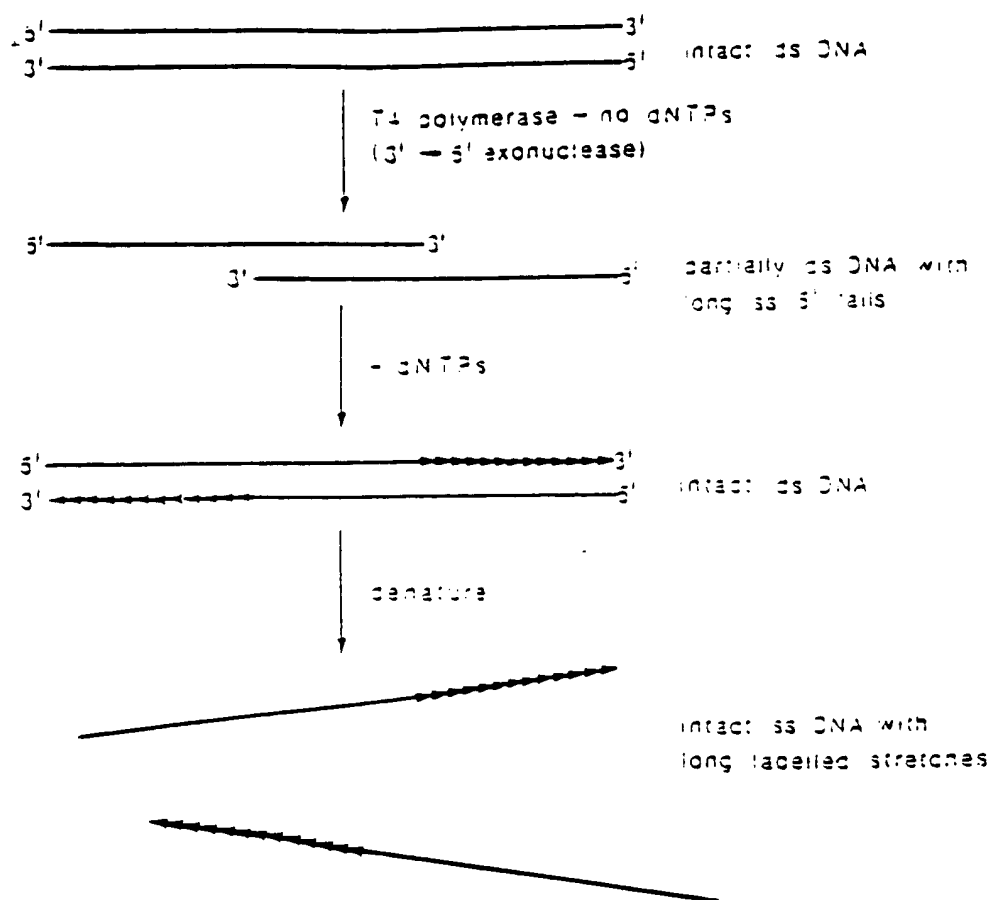


Figure 3. The preparation of probes using T4 DNA polymerase: —, labelled strand; dNTPs: deoxynucleoside triphosphates; ds and ss DNA, double- and single-stranded DNA, respectively.

- (ii) In another microcentrifuge tube, mix:
- 2 μ l 5 x reaction buffer (0.33 M sodium acetate, 50 mM magnesium acetate, 500 μ g/ml BSA, 2.5 mM DTT, 0.165 M Tris-acetate, pH 7.9)
 - 0.2 μ g target DNA
 - 4 μ l sterile distilled water
 - 2 μ l T4 DNA polymerase (0.8 units) as prepared in step (i).
- (iii) Mix, then centrifuge briefly to ensure the contents are at the bottom of the tube.
- (iv) Incubate at 37°C for the length of time needed to remove the required number of nucleotides (25 nucleotides removed per min under these conditions).
- (v) Cool the tube in ice water.
- (vi) Add to the tube:
- 3 μ l 5 x reaction buffer [see step (ii)]
 - 0.5 μ l water
 - 2.5 μ l 2 mM dCTP
 - 2.5 μ l 2 mM dGTP
 - 2.5 μ l 2 mM dTTP
 - 4.0 μ l [α -³²P] dATP (400 Ci/mmol; 10 mCi/ml)
- (vii) Mix thoroughly and centrifuge briefly (as step (iii)).
- (viii) Incubate at 37°C for 25 min.

- (ix) Stop the reaction by adding 5 μ l of 0.3 M EDTA or heating at 65°C for 15 min.
- (x) Separate the labelled DNA from unincorporated nucleotides by chromatography through Sephadex G-50 as in Section 4.1.2. steps (v) and (vi).

The T4 DNA polymerase labelling procedure has several advantages over the nick translation procedure.

- (a) This procedure yields an intact double-stranded molecule with no nicks. It can therefore be cut with restriction enzymes and is more resistant to exonuclease attack.
- (b) Defined regions of the DNA can be labelled by controlling the reaction conditions and thus the length of DNA labelled.
- (c) DNA can be labelled to extremely high specific activity (10^9 d.p.m./ μ g) if high specific activity nucleotides (2000 Ci/mmol) are used. In this case, the volume of the [α - 32 P]dATP used [step (vi)] must be increased to maintain the concentration of dATP at the value given here so that the polymerase will work. The larger volume of [α - 32 P]dATP required must be concentrated by drying *in vacuo* and then resuspended to 4 μ l final volume ready for addition to the reaction mixture [step (vi)]. Although this yields highly radioactive DNA, DNA labelled to this level is unstable, that is, it is cleaved during the radioactive decay.
- (d) Labelling of one strand of a double-stranded DNA fragment can be achieved.

The disadvantages of the method become evident when long stretches of DNA are to be labelled, since secondary structures form in the long single-stranded regions. These secondary structures inhibit the polymerase activity.

4.1.4 End-labelling with T4 Polynucleotide Kinase

Polynucleotide kinase is used to transfer the γ phosphate of ATP to a free 5' OH group in either DNA or RNA. The enzyme also has a phosphatase activity. Two reactions are therefore possible (34,35). In the forward reaction, the enzyme catalyses phosphorylation following removal of 5'-terminal phosphates with alkaline phosphatase. In the exchange reaction, the kinase catalyses the exchange of an existing 5' phosphate with the γ phosphate of ATP. The latter reaction has to be carried out in the presence of excess ATP and ADP if efficient phosphorylation is to be achieved. Both reactions are most efficient with DNA which has a protruding 5' terminus: recessed 5' ends are poorly phosphorylated. Specific activities of 5×10^5 c.p.m./pmol ends and 8×10^5 c.p.m./pmol ends can be achieved with the exchange and forward reactions, respectively, using blunt-ended DNA fragments. RNA is most effectively labelled by T4 polynucleotide kinase following base cleavage as described in Table 7.

The detailed protocols for end-labelling DNA or RNA with T4 polynucleotide kinase are as follows.

Forward reaction.

- (i) Dissolve the DNA fragments (5 pmol of 5' ends) in 100 μ l of 50 mM Tris-HCl pH 8.0 and add 0.5 units of bacterial alkaline phosphatase. Incubate at 65°C for 60 min. Alternatively, for RNA fragments, use 1.5 units of alkaline phosphatase and incubate for 10 min at 45°C.

Table 7. Base Cleavage of RNA to be Labelled with T4 Polynucleotide Kinase.

1. To 200 μ l of RNA at 50 μ g/ml in distilled water in a microcentrifuge tube, add 20 μ l of 1 M NaOH.
2. Leave on ice for 10 min to produce fragments of 200–1000 bases.
3. Add 20 μ l of 1 M Tris-HCl pH 3.0 and sufficient HCl to neutralise the NaOH (check with pH paper).
4. Add 4 μ l of 4 M NaCl plus 500 μ l of ethanol. Leave at -20°C for at least 30 min.
5. Recover the precipitated RNA by centrifugation for 5 min in a microcentrifuge. Resuspend the pellet in distilled water at a concentration of 50 μ g/ml. Store at -20°C .

- (ii) After the incubation, add stock EDTA to a final concentration of 10 mM. Heat at 65°C for 10 min then extract three times with an equal volume of buffer-equilibrated phenol.
- (iii) Add stock NaCl to a final concentration of 50 mM and precipitate the DNA with 2 volumes of ethanol at -20°C overnight or RNA with 3 volumes of ethanol at -20°C overnight.
- (iv) Re-dissolve the nucleic acid in 20 μ l of 15 mM DTT, 10 mM MgCl_2 , 0.33 μ M ATP, 60 mM Tris-HCl, pH 7.3 containing 10 μ Ci [γ - ^{32}P]ATP (>5000 Ci/mmol).
- (v) Add 5 units of polynucleotide kinase and incubate at 37°C for 30–60 min.
- (vi) Remove any unincorporated nucleotides by chromatography through G-50 Sephadex as in Section 4.1.2, steps (v) and (vi).

Exchange reaction.

- (i) Dissolve the DNA or RNA fragments (5 pmol of ends) in 25 μ l of 12 mM MgCl_2 , 1 mM DTT, 0.3 mM ADP, 0.5 μ M ATP, 50 mM imidazole-HCl, pH 6.4.
- (ii) Add 50 μ Ci [γ - ^{32}P]ATP (5000 Ci/mmol) and 5 units of T4 polynucleotide kinase.
- (iii) Incubate at 37°C for 30–60 min.
- (iv) Remove unincorporated nucleotides by chromatography through G-50 Sephadex [Section 4.1.2, steps (v) and (vi)].

The advantages of the use of T4 polynucleotide kinase to label nucleic acids for hybridisation are:

- (a) it can be used to label RNA as well as DNA;
- (b) the exact location of the labelled group is known;
- (c) very small pieces of nucleic acid can be labelled;
- (d) DNA fragments from a restriction enzyme digest can be labelled before separation by gel electrophoresis thus permitting the preparation of several probes at once.

The disadvantage is that the specific activity of the final product depends solely on the specific activity of the [γ - ^{32}P] nucleotide used and cannot be adjusted by adding more enzyme.

4.1.5 End-labelling with Terminal Deoxynucleotidyl Transferase

Terminal deoxynucleotidyl transferase (often called terminal transferase) adds deoxy-ribonucleotides onto the 3' ends of DNA fragments. Both single- and double-stranded DNAs are substrates for this enzyme, and if cobalt ions are present as co-factor, even recessed 3' ends can be used as substrates.

Terminal transferase is useful in two ways:

- (i) to make complementary homopolymer tails on DNA fragments which are to be joined (by annealing and ligation) during a cloning experiment (36);
- (ii) to label the 3' ends of DNA molecules using [α - 32 P]nucleoside triphosphates. Problems caused by the generation of homopolymer tails of random length can be avoided by the use of the nucleotide analogue [α - 32 P]cordycepin triphosphate [3' deoxyadenosine 5' triphosphate (ref. 39)]. The lack of a 3' hydroxyl group on cordycepin triphosphate prevents further nucleotide additions, resulting in chain termination and the generation of DNA molecules labelled by the addition of a single nucleotide only.

The labelling procedure is as follows.

- (i) To the DNA (5 pmol of 3' ends), add 5 μ l (50 μ Ci) [α - 32 P]cordycepin triphosphate (> 3000 Ci/mmol; 10 mCi/ml).
- (ii) Add 10 μ l of 5 x tailing buffer (10 mM CoCl₂, 1 mM DTT, 0.5 M potassium cacodylate, pH 7.2).
- (iii) Adjust the volume to 49 μ l with water and mix well.
- (iv) Add 15 units of terminal transferase (1 μ l). Mix and incubate at 37°C for 30 min.
- (v) Add an equal volume of phenol saturated with 50 mM Tris-HCl, pH 7.5, and mix to stop the reaction. Separate the phases by centrifugation for 5 min in a microcentrifuge.
- (vi) Transfer the upper (aqueous) phase to a new tube and add 0.5 volumes of 7.5 M ammonium acetate followed by 2 volumes of ethanol.
- (vii) Store at -70°C for 15-30 min to precipitate the DNA.
- (viii) Recover the DNA by centrifugation and redissolve the pellet in 50 μ l TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA).
- (ix) Repeat the ethanol precipitation [steps (vi)-(viii)] but air-dry the pellet and dissolve it in the buffer of choice. This procedure removes most of the unincorporated cordycepin. Alternatively the DNA can be purified by chromatography through Sephadex G50 [Section 4.1.2, steps (v) and (vi)].

The advantage of the use of terminal transferase to produce labelled DNA probes is that the molecules are specifically labelled at the 3' ends only. The disadvantage, as for T4 polynucleotide kinase, is that the specific activity of the final product is totally dependent on the specific activity of the [α - 32 P]cordycepin triphosphate.

4.1.6 End-labelling with the Large (Klenow) Fragment of *E. coli* DNA Polymerase I

The large (Klenow) fragment of *E. coli* DNA polymerase I, which has the 5' - 3' polymerase activity, can be used to 'fill in' the 3' ends of DNA fragments opposite naturally-occurring 5' extensions or those produced by some restriction enzymes e.g., *Bam*HI, *Hind*III (38). The reaction is shown diagrammatically in Figure 4 and is described below.

- (i) To 1 μ g of DNA in a common restriction enzyme buffer, such as 6 mM MgCl₂, 1 mM DTT, 50 mM NaCl, 6 mM Tris-HCl, pH 7.5, add 10 μ l of a mixture of unlabelled nucleotides (0.1 mM each). The nucleotides supplied must be correct for the particular 3' end to be filled in, minus the one or more labelled

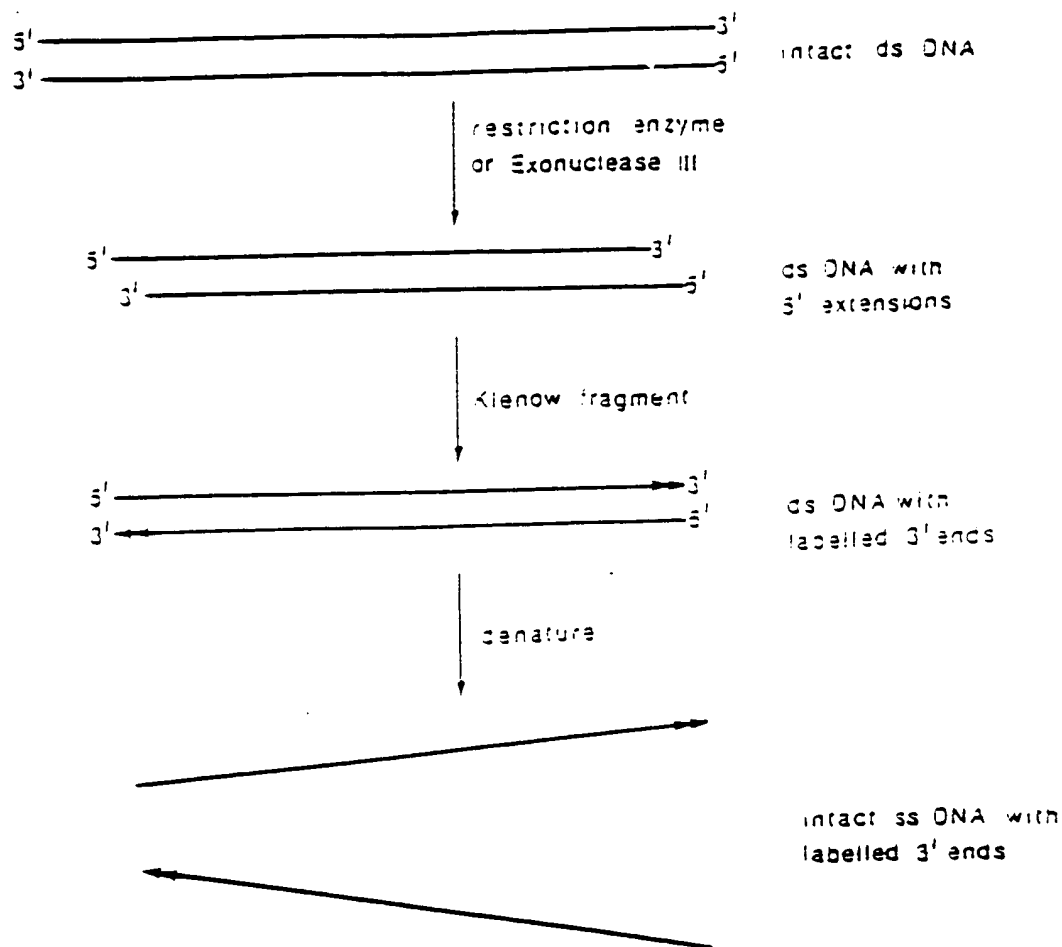


Figure 4. Preparation of probes using the large (Klenow) fragment of *E. coli* DNA polymerase I. \blacktriangleright labelled strand; ds and ss DNA, double- and single-stranded DNA, respectively.

nucleotides to be used. For example, to fill in *Bam*HI 3' termini, which are
 CCTAG
 G

add unlabelled dGTP, dTTP and dCTP if labelled dATP is being used. For very high specific activity probes, omit the unlabelled nucleotides and add only radiolabelled dGTP, dATP, dTTP and dCTP at step (ii).

- (ii) Add the α - 32 P-labelled nucleotide(s) as aqueous solution(s) [2 μ Ci each (> 3000 Ci/mmol)]
- (iii) Adjust the volume to 29 μ l with water. Then add 1 μ l (5 units) of the Klenow fragment of DNA polymerase I.
- (iv) Incubate for 15 min at room temperature.
- (v) Remove unincorporated nucleotides by chromatography through Sephadex G-50 [Section 4.1.2, steps (v) and (vi)].

The advantages of this method are:

- (a) it may be carried out directly after digesting DNA with a restriction enzyme; there is no need to purify the fragment(s)

Preparation of Nucleic Acid Probes

- (b) by adding the appropriate labelled nucleotides, all the 3' ends can be filled completely with stretches of labelled DNA, thus increasing the specific activity of the product.

The disadvantage is that it cannot be used on DNA with 3' overhanging termini. Blunt ends are labelled by the exchange of one base at the 3' OH terminus.

4.1.7 The Use of Exonuclease III to Create 3' Overhangs in DNA Molecules

If it is necessary to end-label DNA which has blunt ends or 3' overhangs, and replacement synthesis is desired, an alternative to T4 DNA polymerase labelling (Section 4.1.3) is to use exonuclease III to create recessed 3' ends which can then be filled in using either the Klenow fragment of DNA polymerase I or AMV reverse transcriptase as previously described (Sections 4.1.6 and 3.2, respectively). The reaction conditions for the use of exonuclease III are as follows:

5 mM MgCl₂
10 mM 2-mercaptoethanol
0.5–1 µg DNA
50 mM Tris-HCl, pH 8.0
Exonuclease III

The reaction with any particular DNA substrate should be optimised by titration using 1–4 units of enzyme for 5–30 min at 37°C.

4.2 Special Cloning Systems for High Specific Activity Radiolabelling of Nucleic Acids

4.2.1 The M13 Universal Probe Primer

High specific activity hybridisation probes can be generated using the M13 universal probe primer (39). The DNA which is to be used as probe should first be cloned into one of the 'even' series of bacteriophage M13 vectors (e.g., M13mp8) by standard techniques for cloning into plasmids (7). Do not use the 'odd' series of M13 vectors (e.g., M13mp9) since this may result in the production of a labelled probe which hybridises with pBR322-derived sequences. The 13-base sequence of the M13 universal probe primer (5'-GAAATTGTTATCC3') is complementary to the 5' side of the multiple cloning site of the family of M13 vectors and is used to initiate synthesis of the (–) strand from the (+) strand template by the Klenow fragment of *E. coli* DNA polymerase I. The synthesis of the complementary strand, which can be labelled by incorporation of an [α -³²P]deoxyribonucleotide, does not proceed to completion so that the inserted probe sequence remains single-stranded. The resulting hybridisation probe, at a specific activity of up to 5×10^8 d.p.m./µg, is therefore single-strand-specific and is used without denaturation for hybridisation analyses. The synthesis of the probe is shown diagrammatically in *Figure 5*.

The conditions used for making labelled probes by this method are much simpler than those used in more conventional labelling methods such as nick translation. A single reaction requires only 2 ng of primer and 50 ng of M13 (+) strand template containing the probe DNA insert, 10 µCi of [α -³²P]dNTP and one unit of the Klenow fragment of DNA polymerase I. A typical reaction is carried out as follows:

3.3 Techniques for Labelling Probes

Detailed protocols for the many techniques available for labelling nucleic acid probes are given in Chapter 2. The following sections describe variations of these techniques used in the author's laboratory specifically to label probes for *in situ* hybridisation.

3.3.1 *In Vitro* Transcription by *E. coli* RNA Polymerase

Both double-stranded DNA and single-stranded DNA (such as M13 clones) can be more-or-less randomly transcribed by *E. coli* RNA polymerase (6). A typical labelling protocol is given in Table 1.

3.3.2 *In Vitro* Transcription with SP6 RNA Polymerase

An efficient way to obtain a single-stranded RNA probe for a known DNA sequence

Table 1. Preparation of Labelled Probes using *E. coli* RNA Polymerase.

| | |
|-----|---|
| 1. | To a microcentrifuge tube, add: 50 μ l [³² P]UTP (50 Ci/mmol) 5 μ l of a mixture of 1 mM ATP, CTP and GTP Dry down under vacuum. |
| 2. | Add to the dried ribonucleotides: 5x salts solution ^a 18.4 μ l 0.125 M MnCl ₂ 1.6 μ l 0.2% 2-mercaptoethanol 20 μ l Cloned DNA 1-2 μ g <i>E. coli</i> RNA polymerase 1-2 units Water to 100 μ l final volume |
| 3. | Incubate at 37°C for 1-2 h. |
| 4. | Add 200 μ l of 10 mM Tris-HCl, pH 7.9, and 10 μ l DNase (1 mg/ml; RNase-free). Leave at room temperature for 10-15 min. |
| 5. | Spot 1 μ l aliquots onto two nitrocellulose filters. Dry one filter. Rinse the other in ice-cold 5% TCA for 5 min, wash in 70% ethanol and dry. Count both filters. The proportion of the radioactivity that is TCA-insoluble will vary but should be at least 10%. The incorporation is usually much higher. |
| 6. | Add 10 μ l 0.2 M EDTA, 20 μ g <i>E. coli</i> tRNA (20 mg/ml), 25 μ l 10% SDS, 300 μ l phenol-chloroform-isoamylalcohol (25:24:1 by vol). Mix by vortexing (1 min). |
| 7. | Centrifuge and remove the top (aqueous) layer into a clean microcentrifuge tube. |
| 8. | Add an equal volume of chloroform:isoamyl alcohol (24:1, v/v). |
| 9. | Centrifuge for 5 min at 10 000 g. Remove the chloroform (bottom layer). |
| 10. | Repeat the chloroform extraction (step 9). |
| 11. | Add 600 μ l ethanol and leave at -20°C for at least 1 h. |
| 12. | Centrifuge for 25 min at 10 000 g. Discard the supernatant. |
| 13. | Dissolve the RNA pellet in distilled water that has been treated with diethylpyrocarbonate (DEPC) ^b . |
| 14. | Bring to 0.3 M ammonium acetate. Add 2.5 volumes of ethanol. Leave at -20°C for at least 1 h. Repeat steps 12 and 13. |
| 15. | Repeat step 14. |
| 16. | Reduce the overall size of the RNA molecules to 50-150 nucleotides by limited alkaline hydrolysis as described in Section 3.2. |
| 17. | Recover the RNA by ethanol precipitation (steps 11 and 12). |
| 18. | Dissolve the RNA in DEPC-treated water using only 75% of the volume required for the hybridisation reaction ^c . |

^a5x Salt solution is prepared by mixing 4 ml of 1 M Tris-HCl (pH 7.9), 19 ml of 1 M KCl, 0.6 ml of 1 M MgCl₂, 0.9 ml of 10 mM EDTA.

^bDistilled water is treated with DEPC to inactivate any contaminating RNase. The procedure is to add DEPC to 0.1% final concentration and then leave at room temperature for 12 h. Residual DEPC is then destroyed by autoclaving the solution for 15 min.

^cIf the hybridisation buffer is to contain formamide, the RNA should be dissolved in a correspondingly smaller volume of water.

In situ Hybridisation

Table 2. Preparation of Labelled Probes using SP6 Polymerase^a.

1. Dry down 50 μ Ci [³H]UTP (50 Ci/mmol) in a disposable plastic microcentrifuge tube.
2. At room temperature, add:

| | |
|--|-------------|
| 5 x transcription buffer ^b | 4 μ l |
| 1 mM each of ATP, CTP and GTP ^c | 4 μ l |
| 0.20 M DTT ^c | 1 μ l |
| Bovine serum albumin (sterile: 2 mg/ml) ^c | 1 μ l |
| RNasin (Promega Biotech) | 20 units |
| Cloned DNA ^d | 1-3 μ g |
| SP6 polymerase | 5 units |

 DEPC-treated water to 20 μ l final volume.
3. Incubate for 1 h at 40°C.
4. Add 75 μ l DEPC-treated water, 40 units DNase (RNase-free: Worthington) and 75 units RNasin. Incubate for 10 min at 37°C.
5. Estimate the incorporation of radioactivity by determining the TCA-precipitable counts as in Table 1, step 5.
6. Extract the mixture with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1 by vol.). Mix by vortexing for 1 min. Separate the phases by centrifugation.
7. Extract the upper (aqueous phase) twice with chloroform.
8. Add 50 μ g yeast tRNA as carrier and then add stock ammonium acetate to 0.7 M final concentration.
9. Add 2.5 volumes of ethanol and recover the RNA as in Table 1, steps 11 and 12.
10. Redissolve the RNA in DEPC-treated water and repeat the precipitation (steps 8 and 9) twice more but without adding more yeast tRNA carrier.
11. Finally, dissolve the RNA pellet in DEPC-treated water. Reduce the size of the RNA to 50-150 nucleotides by limited alkaline hydrolysis as described in Section 3.2.

^aAn alternative procedure for labelling probes using SP6 polymerase is given in Chapter 2, Section 4.2.2.

^bThe composition of 5x transcription buffer is 0.2 M Tris-HCl (pH 7.5), 30 mM MgCl₂, 10 mM spermidine. This buffer is autoclaved and then stored at -20°C.

^cThese solutions are made using DEPC-treated water (see Table 1, footnote b).

^dCircular DNA should be dialysed against 1 mM EDTA, 10 mM Tris-HCl, pH 7.5, to remove salts and then linearised using an appropriate restriction enzyme.

Table 3. Preparation of Labelled Probes by Nick Translation^a.

1. Dry down 150 pmoles of [³H]TTP and 150 pmoles of [³H]dATP in a plastic microcentrifuge tube.
2. Add:

| | |
|---|-------------|
| 0.5 mM dGTP | 1 μ l |
| 0.5 mM dCTP | 1 μ l |
| 10 x salts solution ^b | 1 μ l |
| 1% 2-mercaptoethanol | 1 μ l |
| Cloned DNA | 0.1 μ g |
| DNase I ^c | 1 μ l |
| <i>E. coli</i> DNA polymerase I (10 uni/ μ l) | 1 μ l |

 Water to 10 μ l final volume
3. Incubate the mixture at 14°C for 1 to 2 h.
4. Add 90 μ g of carrier DNA^d and adjust the total volume to 100 μ l with water.
5. Add 3 μ l of 0.1 M spermine. Mix and leave on ice for 15 min.
6. Centrifuge at 10 000 g for 10 min. Discard the supernatant.
7. Resuspend the pellet in 75% ethanol containing 0.3 M sodium acetate and 10 mM magnesium acetate. Vortex. Leave on ice for 1 h, mixing frequently.
8. Centrifuge for 10 min (10 000 g) to recover the DNA.
9. Resuspend the labelled double-stranded DNA probe in water.

^aAn alternative protocol for preparation of labelled DNA probes by nick translation is given in Chapter 2, Section 4.1.2.

^bThe composition of 10 x salts solution for nick translation is 0.5 M Tris-HCl (pH 7.8), 50 mM MgCl₂, 0.5 mg/ml BSA (nucleic acid grade).

^cCommercial DNase I (1 mg/ml stock) is stored in aliquots at -20°C. Before use, the DNase is diluted by a factor of 10³ in water.

^dUse *E. coli* DNA sheared by sonication and denatured by boiling for 10 min.

is to clone the sequence adjacent to a promoter of the phage SP6 (7.3). The SP6 RNA polymerase will only transcribe DNA downstream from this promoter. The end-point of the transcription can be set by cleaving the DNA template with an appropriate restriction enzyme. When preparing a probe for hybridisation to cellular RNA, the promoter should be placed on the non-coding strand of DNA, that is, the strand not transcribed *in vivo*. SP6 polymerase and cloning vectors are commercially available. A typical protocol for labelling probes using SP6 RNA polymerase is given in Table 2.

3.3.3 Nick Translation by *E. coli* DNA Polymerase I

Radioactive nucleotides can be introduced into double-stranded DNA by nick-translation with *E. coli* DNA polymerase I. Starting at a nick in the DNA, the enzyme removes nucleotides from the 5' side of the nick while adding nucleotides to the 3' side. If this replacement synthesis is carried on in the presence of radioactive precursors, the DNA can be labelled to a high specific radioactivity (9,10). The amount of replacement synthesis is controlled, to a large extent, by the number of nicks which provide entry for the polymerase. The number of nicks depends on the amount of DNase I present in the reaction (or nicks introduced during DNA isolation). A typical protocol is given in Table 3.

3.3.4 Notes on the Specific Radioactivity of Probes

The specific radioactivity of probes synthesised by RNA polymerases is limited only by the specific activity of the nucleotide precursors. For hybridisation to clustered repeated DNA, to polytene chromosomes, or to an abundant RNA, a probe labelled with only [³H]UTP (50 Ci/mmol) is sufficient. If the target of hybridisation is smaller, it is possible to increase the specific radioactivity of the probe by replacing one or more of the other unlabelled nucleoside triphosphates with additional ³H-labelled nucleoside triphosphates.

The specific radioactivity of DNA labelled by nick translation depends both on the nucleotide precursors and on the extent of replacement of unlabelled DNA. The typical nick-translation reaction described in Table 3 gives probes labelled to about 3×10^7 c.p.m./ μ g using [³H]TTP (80 Ci/mmol) and [³H]dATP (14 Ci/mmol). This allows detection of sequences in polytene chromosomes corresponding to a typical phage λ clone in 1–3 days.

The amount of hybrid detected depends both on the specific activity of the probe and on how many of the target sequences have bound the probe. Therefore, it is useful to use the probe at a concentration that will nearly saturate the target DNA. Higher concentrations of probe will only contribute to the background without improving the signal. For hybridisation to polytene chromosomes and to other abundant sequences, the probe is usually used at well below saturating concentrations for reasons of economy. With such large targets, hybrids can be detected after only a few days of autoradiographic exposure even if the DNA is not completely saturated.

4. HYBRIDISATION TO DNA IN CYTOLOGICAL PREPARATIONS

4.1 Cytological Procedures

It is not possible to design a single procedure that makes optimal preparations from

Decisions of the United States Courts and of the United States Patent and Trademark Office in Patent, Trademark, and Copyright Cases

Court of Appeals, Eighth Circuit

E.I. duPont de Nemours & Company
v. Berkley & Company, Inc.

Nos. 79-1219 and 79-1231
Decided Feb. 13, 1980

PATENTS

1. Costs — In general (§25.1)

Costs — Attorney's fees (§25.5)

Award of fees and costs is available only to prevailing party.

2. Pleading and practice in courts — Findings of fact and conclusions of law (§53.40)

Pleading and practice in courts — Jury trial — In general (§53.571)

Jury is not required to make extensive factual findings on question of patent invalidity required of judge under Fed.R.Civ.P. 52(a), although more detailed findings would have been preferable.

3. Patentability — Utility (§51.75)

Lack of utility means invention is incapable of achieving any of aims of patent under any conditions.

4. Estoppel — As to validity — In general (§35.151)

Patentability — Evidence of — Commercial success — In general (§51.4551)

Patentability — Evidence of — Infringement (§51.463)

Patentability — Utility (§51.75)

Presumption from patent grant — In general (§55.1)

One who appropriates teachings of patent may not deny utility of invention; accused infringer's admission that its device fell within scope of patent's claims is admission of utility in invention there claimed, and it is thereby estopped from asserting that those claims are invalid for lack of utility; presumption of utility is strengthened when invention is copied and becomes commercial success.

5. Patentability — Evidence of — Commercial success — In general (§51.4551)

Patentability — Evidence of — Infringement (§51.463)

Patentability — Utility (§51.75)

Presumption from patent grant — In general (§55.1)

Presumption of utility created by issuance of patent is strengthened when others have copied invention and it has achieved commercial success.

6. Construction of specification and claims — Claim defines invention (§22.30)

Claims measure invention, but invention must be viewed as a whole; claims must be read in their entirety.

7. Operability (§48)

Patentability — Utility (§51.75)

Pleading and practice in courts — Burden of proof — Validity (§53.138)

Small degree of utility is sufficient; claimed invention must only be capable of performing some beneficial function; perfection under all conditions is not required, whether patent does or does not suggest that invention is imperfect or inoperable under certain conditions; inventions does not lack utility merely because particular embodiment disclosed in patent lacks perfection or performs crudely; commercially successful product is not required, nor is it essential that invention accomplish all its intended functions or operate under all conditions, partial success being sufficient to demonstrate patentable utility; defense of non-utility cannot be sustained without proof of total incapacity; proof of inoperativeness or non-utility must be strong, every reasonable doubt being resolved in favor of patentee.

8. Patentability — Utility (§51.75)

Pleading and practice in courts — Jury trial — In general (§53.751)

Submission to jury, for any purpose, of issue of whether patent was invalid for lack of utility, which should not have gone to jury, but should have been decided in patent owner's favor as matter of law, was prejudicial error.

9. Patentability — Anticipation — Prior knowledge, use or sale (§51.223)

Pleading and practice in courts — Burden of proof — In general (§53.131)

Use and sale — Character of evidence to prove (§69.3)

Patent is invalid under 35 U.S.C. 102(a) or 102(b) if claimed invention was known or used by others in this country prior to patentee's invention, or was in public use or on sale in this country more than one year before patent application was filed; burden of proof of prior use is satisfied only by evidence clear, cogent, and convincing.

10. Evidence — Weight and credibility (§36.40)

Use and sale — Character of evidence to prove (§69.3)

More widespread view is that unsupported oral testimony of prior use can be sufficient, but must be regarded with suspicion and subjected to close scrutiny; delay between event and trial, witness' interest, contradiction or impeachment, corroboration, witness' familiarity with details of alleged prior structure, improbability of prior use considering state of art, impact of invention on industry, and relationship between witness and alleged prior user, are factors considered in determining sufficiency of oral testimony.

11. Evidence — Weight and credibility (§36.40)

Patentability — Anticipation — Prior knowledge, use or sale (§51.223)

Pleading and practice in courts — Jury trial — In general (§53.571)

Uncorroborated oral testimony concerning events of long ago, contradicted by alleged prior user himself and by unchallenged results of scientific tests, had insufficient probative value to make out case of prior use that jury could have found clear and convincing.

12. Patentability — Invention — In general (§51.501)

Patentability — Tests of — In general (§51.701)

Determination of obviousness issue is governed by principles articulated by Supreme Court in *Graham v. John Deere Co.*, 148 USPQ 459.

13. Courts of Appeals — Issues determined (§29.10)

Patentability — Invention — In general (§51.501)

Ultimate question of obviousness is subject to evaluation on appeal; sounder procedure is for initial obviousness determination to be made by trial court.

14. Patentability — Invention — Law or fact question (§51.507)

Pleading and practice in courts — Jury trial — In general (§53.751)

Obviousness-nonobviousness conclusion rests on factual inquiries to be resolved in first instance by jury in jury trial.

15. Evidence — In general (§36.01)

It would be unjust, unfair, and unjudicial to ignore any unforbidden evidence relative to ob-

viousness-nonobviousness question.

16. Patentability — Invention — In general (§51.501)

Pleading and practice in courts — Trial (§53.80)

Parties are entitled to consideration in first instance of all facts touching on obviousness issue, by factfinder who sees and hears witnesses.

17. Patentability — Anticipation — In general (§51.201)

Patentability — Anticipation — Prior knowledge, use or sale (§51.223)

Use and sale — In general (§69.1)

Prior use or sale constitutes "prior art" only if it occurs in this country.

18. Patentability — Anticipation — In general (§51.201)

Patentability — Evidence of — State of art (§51.467)

Patentability — Tests of — Skill of art (§51.707)

Pleading and practice in courts — Jury trial — In general (§53.571)

Instruction that jury consider foreign product "solely as it bears on the state of the art and as it relates to the ultimate question of obviousness" supplied no guidance from which jury could be expected to have distinguished foreign product from prior art and, considered as whole, was thus misleading and erroneous; possibility of jury confusing evidence admitted to show "state of the art" or "skill in the art" with other evidence admitted to show "prior art" weighs heavily against use of cautionary instruction phrased in terms employed here.

19. Patentability — Evidence of — Solution by several parties (§51.465)

Patentability — Invention — In general (§51.501)

Pleading and practice in courts — Jury trial — In general (§53.571)

Similarity between contemporaneous invention and prior art dictates that evidence of former be cautiously admitted in jury trial, and trial judge must carefully instruct jury that such evidence is merely one possible indicium of obviousness; same patentable invention may be contemporaneously made by more than one inventor, and fact that invention was contemporaneously

made elsewhere may or may not, in light of all circumstances, be some indication that invention would have been obvious to one of ordinary skill in art.

20. Interference — In general (§41.01)

Patent grant — Intent of patent laws (§50.15)

Patentability — Evidence of — Solution by several parties (§51.465)

35 U.S.C. 135, establishing interferences in Patent and Trademark Office, is entirely premised on concept that same nonobvious invention may be contemporaneously made by plurality of inventors; congressional mandate, and priority status given in interferences to first-to-file patent application, accord with Constitutional purpose of patent system, which is, to encourage public disclosure of inventions in this country.

21. Pleading and practice in courts — Burden of proof — Validity (§53.138)

Presumption from patent grant — Patent Office consideration of prior art (§55.5)

Patent is presumed valid under 35 U.S.C. 282 and burden of establishing invalidity rests on party asserting it; statutory presumption of validity flows from congressional assumption that Patent and Trademark Office properly performs its administrative functions; mandate of section 282 is twofold, requiring that party asserting invalidity bear not only presumption — generated burden of going forward with proof but also burden of persuasion on that issue; latter burden remains upon party asserting invalidity whether relevant prior art was or was not considered by examiner during prosecution of patent application; presumption is not conclusive and can be rebutted by proof that Patent and Trademark Office erred; presumption is fully rebutted only when party asserting invalidity meets burden of persuasion, that is, relies on evidence that does in truth render claimed invention invalid, though rebuttal may be easier when prior art relied on is more relevant than that considered by examiner; court or jury is not precluded from being convinced that patent should have been refused in view of reference cited or assumedly considered, and presumption of validity is not limited to those references actually cited by examiner.

22. Construction of specification and claims — By Patent Office proceedings — In general (§22.151)

Pleading and practice in Patent Office — In general (§54.1)

Pleading and practice in Patent Office — Rules effect (§54.9)

Presumption from patent grant — Patent Office consideration of prior art (§55.5)

Function of Patent and Trademark Office entails thorough scrutiny of prior art references; rules of practice requires examiner to cite only what he considers "best references"; file wrapper, or prosecution history, of patent application lists references cited against application and classes and subclasses of references inspected by examiner; examiner's search record is prima facie evidence that he considered all references classified in classes and subclasses searched and that he left uncited those he regarded as less relevant than those cited; contrary view would destroy presumption of administrative regularity on which presumption of validity rests.

23. Pleading and practice in courts — Jury trial — In general (§53.571)

Presumption from patent grant — Patent Office consideration of prior art (§55.5)

Congress, in enacting presumption of validity, chose to assume prima facie that oversight of relevant prior art did not occur; it is improper to conclude that references classified in areas searched by examiner, but not specifically cited by him, were not considered; trial court's instruction that "presumption does not extend or exist as to prior art patents or publications which do not appear from record of file wrapper," left jury unappraised of full extent of statutory presumption of validity.

24. Pleading and practice in courts — Jury trial — In general (§53.571)

Presumption from patent grant — Patent Office consideration of prior art (§55.5)

Refusal of trial court to give requested instruction that "it is also assumed that the examiner reviewed the prior art which was in the files which he searched during the prosecution of the application for the patent" together with instruction that "presumption does not extend or exist as to prior art patents or publications which do not appear from the record of the file wrapper" left jury unappraised of full extent of statutory presumption of patent validity, and, while instructions must be considered

in their entirety, court's reference in same sentence of instruction to patents that "had not been considered . . . if they are more pertinent than those considered" could not cure potential for jury's misdirection.

25. Defenses — In general (§30.01)

Evidence — Expert testimony (§36.10)

Fact that accused infringer's expert took certain view of scientific report at trial does not establish gross negligence in patent owner's failure to take same view before or after trial.

26. Defenses — In general (§30.01)

Pleading and practice in courts — Jury trial — In general (§53.571)

Notion that patent owner could be found guilty of gross negligence in accepting its patent and exercising its legal right to seek legal enforcement in courts, for failure to anticipate jury verdict of invalidity is meritless.

27. Evidence — In general (§36.01)

Pleading and practice in courts — Jury trial — In general (§53.571)

Recognition that infringement suit may not succeed simply reflects realistic awareness of historically low percentage of patents held valid in litigation; potential for jury misconstruction of exhibits reflecting internal consideration of cost versus bringing suit, which is legitimate consideration for any patentee to engage in, would have been unacceptably prejudicial.

PATENTS

28. Misuse of patents — In general (§45.01)

Misuse of patents — Threats to sue (§45.55)

UNFAIR COMPETITION

Antitrust laws (§68.15)

Every good faith effort to enforce patent involves legitimate anticompetitive intent, by definition; if patent be valid, competition involves product that by definition did not exist before inventor contributed it to marketplace, and enforcement of patent takes nothing from public, and nothing from infringer to which it had any right; there is no legal obligation of patentees to withhold suit until infringer is well along in its infringement.

UNFAIR COMPETITION

29. Antitrust laws (§68.15)

There is no merit to argument that enforcement of patent with anticompetitive in-

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tent would alone support antitrust claim, even if patent owner had no actual knowledge of invalidity and was not guilty of gross negligence in failing to determine invalidity.

30. Antitrust laws (§68.15)

Antitrust counterclaim may not be based on "inequitable conduct"; enforcement of patent procured by fraud on Patent and Trademark Office may violate Sherman Act Section 2 if other elements necessary to Section 2 case are present; it is not as much violation of Sherman Act for patent owner to enforce "unenforceable" patent against potential competitors as it is to enforce "invalid" patent.

PATENTS

31. Defenses — Fraud (§30.05)

UNFAIR COMPETITION

Antitrust laws (§68.15)

Patent procured by fraud, by definition, would not have issued but for misrepresentation or non-disclosure; such patent is invalid as improperly issued, and patentee has illegally received exclusionary rights he would not otherwise have, so that severe sanctions of Sherman Act may be warranted; patent that was not procured through non-disclosure would properly issue and patentee would receive no exclusionary rights to which he was not legally entitled under patent laws, and no basis would exist for charge of illegal monopolization or attempt to monopolize.

UNFAIR COMPETITION

32. Antitrust laws (§68.15)

Inequitable conduct combined with anticompetitive intent in bringing suit on patent is insufficient basis for antitrust cause of action; exhibits that focus exclusively on events occurring after issuance of patent bear no relation to patentee's conduct before Patent and Trademark Office, and cannot bootstrap non-fraudulent conduct, not otherwise actionable under antitrust laws, into more egregious type conduct of Walker Process Equipment, Inc. v. Food Machinery & Chemical Corp., 147 USPQ 404.

33. Defenses — Unclean hands (§30.25)

Pleading and practice in courts — Jury trial — In general (§53.571)

Absent ruling that evidence was insufficient, or more prejudicial than probative, instruction on inequitable conduct as defense of non-enforceability would be proper.

34. Defense — Unclean hands (§30.25)

Pleading and practice in courts — In general (§53.01)

Expressly pled fraud allegation that differed from inequitable conduct theory only in degree of materiality of information allegedly withheld from Patent Office, placed patent owner on notice of type of conduct that would be litigated, which is all that is required.

PATENTS

35. Defenses — Unclean hands (§30.25)

Inequitable conduct short of fraud can be defense in patent infringement suit; to make out case of inequitable conduct, accused infringer must prove, by clear, unequivocal, and convincing evidence, that patentee's conduct made it impossible for Patent and Trademark Office to fairly assess patent application against prevailing statutory criteria, and that it involved some element of wrongfulness, willfulness, or bad faith; innocent or negligent misrepresentation or non-disclosure of irrelevant information does not amount to inequitable conduct, whether or not intentional.

36. Attorneys — Propriety of conduct (§17.7)

Defenses — Fraud (§30.05)

Accused infringer in complex litigation should not be permitted to sidestep main issues by nit-picking patent file in every minute respect with effect of trying patentee personally, rather than patent; patentee's oversights are easily magnified out of proportion by one accused of infringement seeking to escape reach of patent by hostilely combing inventor's files in liberal pretrial discovery proceedings; unjustified damage to professional and social reputations can result, without fostering any corresponding public benefit in form of inhibiting future improvident grants of patent monopolies.

37. Defenses — Fraud (§30.05)

Defenses — Unclean hands (§30.25)

Pleading and practice in courts — Jury trial — In general (§53.571)

Accused infringer has right to jury determination of whether information not disclosed to Patent and Trademark Office by patentee was sufficiently relevant to meet inequitable conduct standard of materiality but not sufficiently relevant to meet fraud standard of materiality; whether information not disclosed was irrelevant or less relevant than that which was disclosed, and

absence of evidence of bad faith, are matters for trial court in first instance or for jury following proper instructions, federal district court properly responded to jury's inquiry as to whether fraud finding would "open the door for a fraud case" against patent owner by stating that jury should ignore consequences of its findings.

Particular patents — Fishing Line

3,063,189, Keller, Fishing Line, holding of invalidity vacated.

Appeal from District Court for Northern District of Iowa, McManus, Ch.J.; 204 USPQ 594.

Action by E. I. duPont de Nemours & Company, against Berkley & Company, Inc., for patent infringement, in which defendant counterclaims for antitrust violations. From judgment holding plaintiff's patent invalid, dismissing counterclaim, and denying defendant's motion for attorney's fees and costs, both parties appeal. Reversed in part.

John O. Tramontine, and Fish & Neave, both of New York, N.Y. (Charles R. Wolle, and Shull, Marshall & Marks, both of Sioux City, Iowa, and Robert C. Morgan and Christopher K. Hu, both of New York, N.Y., on the brief) for E. I. duPont de Nemours & Company.

Dennis W. Johnson and David W. Belin, both of Des Moines, Iowa (Orrin M. Haugen, Thomas Nikolai, and Haugen & Nikolai, both of Minneapolis, Minn., Belin, Harris, Helmick & Lovrien, Des Moines, Iowa, and William J. Rawlings, and Kindig, Beebe, McCluhan, Rawlings & Nieland, both of Sioux City, Iowa, on the brief) for Berkley & Company, Inc.

Before Bright, and Henley, Circuit Judges, and Markey, Chief Judge*.

Markey, Chief Judge.

[1] E. I. du Pont de Nemours & Company (DuPont) appeals from a judgment holding invalid its United States patent No. 3,063,189 (DuPont patent). Berkley & Company, Inc. (Berkley) appeals the dismissal of its antitrust counterclaim and denial of its motion for attorney's fees and costs. We reverse the judgment of invalidity, remand for a new trial on that issue, and affirm the dismissal of the counterclaim.¹

* The Honorable Howard T. Markey, Chief Judge, United States Court of Customs and Patent Appeals, sitting by designation.

¹ An award of fees and costs being available only to a prevailing party, 35 U.S.C. §285 (1976);

Background

The patent in suit, like most patents, discloses a proposed solution for a problem. When colored fishing lines are used, to enable the fisherman to see them above water, the lines are thought to be visible underwater to the fish.² When transparent lines are used, to limit visibility to fish, the fisherman can't see the lines resulting in tangled lines when several are used from one boat.

The solution proposed by Ed Keller, DuPont's employee, was a fishing line containing fluorescent dye. The dye would be activated, i.e., the line would glow, in response to the ultraviolet component of daylight, making it "visible above water." At the same time, because water would absorb the dye-activating ultraviolet component of daylight, the line would be "relatively invisible" below the water. Thus one line would have the characteristics of high visibility above water and low visibility below water. Keller filed a U.S. patent application on January 2, 1962. DuPont introduced its fluorescent dyed line to the marketplace in August, 1962. The DuPont patent issued on November 13, 1962³ to DuPont, Keller's assignee.

In November 1962, Berkley began making and selling a fluorescent fishing line, but ceased in 1963 when DuPont gave notice of infringement. Berkley's subsequent efforts to produce a "High Visibility" line that would not infringe were unsuccessful, its use of anthranilic acid producing a line half as bright as DuPont's. In December 1974, after unproductive licensing discussions with DuPont, Berkley again began making and selling a fluorescent fishing line.

On August 1, 1975, DuPont sued Berkley for willful infringement of claims 1, 2, 5, 6 and 8 of the DuPont patent. Berkley denied infringement throughout a three-year discovery period, asserting that its line contained an "optical brightener" in place of fluorescent dye. Two weeks before trial, Berkley admitted that claims 2, 5 and 6, if valid, were infringed by its product. DuPont thereupon deleted claims 1 and 8 from its charge of infringement.

For its defense, Berkley alleged (1) that the DuPont patent was invalid for lack of

Fed. R. Civ. P. 54(d), consideration of Berkley's claim of error in denial of its attorney's fees and costs in defending the infringement suit is premature. Berkley may renew its claim for attorneys fees and costs if it prevails on retrial.

² The record is silent on how and what fish see.

³ The patent expired on November 13, 1979.

utility, novelty and nonobviousness, and (2) that the patent was rendered invalid by DuPont's fraudulent conduct before the U.S. Patent and Trademark Office (PTO).

For its counterclaim, Berkley alleged that DuPont violated Section 2 of the Sherman Act by procuring the patent through fraud and by attempting to enforce the patent with knowledge of its invalidity.⁴

Over Berkley's objection, the trial court ordered a bifurcated trial, the first part to include the allegations that DuPont obtained the patent by fraud and enforced it believing it invalid. The second part, on the remaining elements of the antitrust claim, would be held if the jury found for Berkley on fraud or enforcement.

[2] Following a three-week trial, the jury returned a general verdict for Berkley on DuPont's infringement claim. The jury answered these, and only these, special interrogatories:⁵

Q: Did plaintiff obtain the * * * [DuPont] patent from the Patent Office by fraud?

A: No.

Q: Did plaintiff assert its patent against Berkley knowing that it was invalid?

A: No.

Q: Did you find for defendant solely because the patent was obvious?

A: No.

The court entered judgment, holding the DuPont patent invalid and dismissing the

⁴ Berkley mistakenly says it filed its counterclaim "promptly" upon discovering the bases therefore. All of those bases were known to it at least 18 months before it filed its counterclaim. The magistrate described timeliness of the filing as "extremely tenuous."

⁵ The court refused DuPont's request for specific interrogatories requiring the jury's grounds for its verdict of invalidity. Though detailed jury findings would have been preferable, and might have avoided a retrial in this case, the refusal was not itself reversible error. A jury is not required to make the extensive factual findings required of a judge under Fed. R. Civ. P. 52(a). *Panther Pumps & Equipment Co. v. Hydrocraft, Inc.*, 468 F.2d 225, 227-28, 175 USPQ 577, 578-580 (7th Cir. 1972), cert. denied, 411 U.S. 965, 177 USPQ 545 (1973).

Whatever the considerations and concerns involved in current discussions of juries in complex litigation, use of interrogatories and special verdicts, from which the parties and an appellate court may glean the basis for the verdict, would appear to alleviate at least some of those concerns in some cases. The trial court's denial of DuPont's request for special verdicts in this case, for example, has forced this court to review every possible basis for the jury's verdict.

complaint and counterclaim. Both parties filed, and the court denied, motions for judgment notwithstanding the verdict or for a new trial.

Issues

Embittered in battle below, the parties request this court to resolve over 25 issues and subissues. The trial court is directly charged with 11 reversible errors. Couched in accusatory and turgid terminology, the briefs set forth numerous bits and pieces of conflicting testimony and documentary evidence, from which we are asked to draw a plethora of factual inferences. The effect is neither a prejudicing of this court, against either side, nor a simplifying clarification. The result is a necessarily extended opinion, based on a searching review of an entire 4000 page record, and in which the issues treated will appear in section headings.⁶

Opinion

I. Validity

A. Requirement for Retrial

Absent error affecting the substantial rights of the parties, neither reversal nor a new trial is required. 28 U.S.C. §2111 (1976); Fed. R. Civ. P. 61.⁷ When the error

⁶ The briefs contain repeated charges and countercharges of factual misstatements, each requiring resolution. An example of unnecessary burden-making is a mischaracterization and misquote of a prior Court opinion, followed not by a phone call and immediate scratching of the quote, but by a strong attack, an apology and explanation, an attack on the explanation, and final assertion that the source is unknown. The limit-lines of zeal are not always clear, but the judicial process is not aided by unnecessary forays into causes of carelessness.

⁷ 28 U.S.C. §2111 (1976) provides:

§2111. Harmless error

On the hearing of any appeal or writ of certiorari in any case, the court shall give judgment after an examination of the record without regard to errors or defects which do not affect the substantial rights of the parties.

Fed. R. Civ. P. 61 provides:

Rule 61. Harmless Error

No error in either the admission or the exclusion of evidence and no error or defect in any ruling or order or in anything done or omitted by the court or by any of the parties is ground for granting a new trial or for setting aside a verdict or for vacating, modifying, or otherwise disturbing a judgment or order, unless refusal to take such action appears to the court inconsistent with substantial justice. The court at every stage of the proceeding must disregard

misled the jury or had a probable effect on its verdict, reversal and a new trial are appropriate. *International Merger & Acquisition Consultants, Inc. v. Armac Enterprises, Inc.*, 531 F.2d 821, 823 (7th Cir. 1976); *Conway v. Chemical Leaman Tank Lines, Inc.*, 525 F.2d 927, 929-30 (5th Cir. 1976); *Hoffman v. Sterling Drug, Inc.*, 485 F.2d 132, 140 (3d Cir. 1973); See *Kotteakos v. United States*, 328 U.S. 750, 764-65 (1946).

Respecting the issue of patent validity under the statute, the court asked the jury to state only whether it found for Berkley "solely" on obviousness. The jury's "No" answer means that there were six possible bases for its verdict: (1) the invention would have been obvious *and* was lacking in utility, (2) the invention would have been obvious *and* was lacking in novelty, (3) the invention would have been obvious *and* was lacking in both utility and novelty, (4) the invention would not have been obvious *but* was lacking in utility, (5) the invention would not have been obvious, *but* was lacking in novelty, or (6) the invention would not have been obvious, *but* was lacking in novelty and utility.

If one or more substantial grounds for the verdict presented no jury question and should have been decided as a matter of law in DuPont's favor, reversal and a new trial are required, notwithstanding the presence of other grounds that could have supported the verdict. *Morrissey v. National Maritime Union*, 544 F.2d 19, 26-27 (2d Cir. 1976); *Albergo v. Reading Co.*, 372 F.2d 83, 85-86 (3d Cir. 1966), cert. denied, 386 U.S. 983 (1967); *Fatovic v. Nederlandsch-Ameridaansche Stoomvaart*, 275 F.2d 188, 190 (2d Cir. 1960); *North American Graphite Corp. v. Allan*, 184 F.2d 387, 389 (D.C. Cir. 1950); *Traveler's Insurance Co. v. Wilkes*, 76 F.2d 701, 705 (5th Cir.), cert. denied, 296 U.S. 604 (1935); *Patton v. Wells*, 121 F. 337, 340 (8th Cir. 1903). See also, *Sunkist v. Winckler & Smith Co.*, 370 U.S. 19, 29-30 (1962) (erroneous instruction); *United Pilots Ass'n v. Halecki*, 358 U.S. 613, 619 (1959) (failure to submit jury question); *Maryland v. Baldwin*, 112 U.S. 490, 493 (1884) (admission of evidence). The utility issue presented no jury question and should have been decided as a matter of law in DuPont's favor. Its submission to the jury, in the circumstances of this case, requires a retrial of the validity issue.⁸

any error or defect in the proceeding which does not affect the substantial rights of the parties.

⁸ A new trial will not result where it is reasonably certain that the jury was not

Berkley concentrated its briefs and oral argument on non-utility, fraud, knowing enforcement of an invalid patent, gross negligence, inequitable conduct, and anticompetitive intent, believing, says Berkley, that that evidence shows the patent invalid. Having viewed all of the evidence on validity most favorably to Berkley, and having given Berkley the benefit of every reasonable inference, *Davis v. Burlington Northern, Inc.*, 541 F.2d 182, 186 (8th Cir.), cert. denied, 429 U.S. 1002 (1976), we cannot say on this record, and in view of the errors occurring below, that Berkley carried its burden of proving the DuPont patent invalid.⁹ DuPont argues for a judgment that its patent is valid. For the reasons discussed *infra*, we decline to enter that judgment on this appeal. To avoid a potential miscarriage of justice, *Firemans Fund Insurance Co. v. Aalco Wrecking Co., Inc.*, 466 F.2d 179, 187 (8th Cir. 1972), we remand for retrial of the validity issue.

B. Utility

[3] Though the trial court instructed the jury that an invention must "operate or function in the manner claimed," and also that an invention "need not work perfectly in order to be useful," the treatment of the utility issue at trial, beyond any error in instructions thereon,¹⁰ was fatally flawed.

The complaint originally asserted infringement of five claims:

1. A fishing line, formed of a synthetic plastic material, which is oriented at least to a point such that a stretching of 100% would cause the line to break, which line

significantly influenced by issues erroneously submitted to it. See *Gardner v. General Motors Corp.*, 507 F.2d 525, 529 (10th Cir. 1974); *Collum v. Butler*, 421 F.2d 1257, 1260 (7th Cir. 1970); *Roginsky v. Richardson-Merrell, Inc.*, 378 F.2d 832, 837-38 (2d Cir. 1967). For the reasons set forth in the text, no such reasonable certainty is possible here.

⁹ In objecting to DuPont's request for interrogatories requiring the jury to state the basis of its verdict, counsel for Berkley stated "It is Berkley's problem to support the verdict in the Court of Appeals, and if there is an erroneous example as to any aspect of the case, that's going to be Berkley's problem. If there is no evidence on some part of the case that went to the jury, that will be Berkley's problem."

¹⁰ DuPont's requested instruction, i.e., that lack of utility means the invention is "incapable of achieving any of the aims of the patent under any conditions," expresses the legal test for utility more clearly and precisely than that given by the trial court. *Infra* note 17.

contains a fluorescent dyestuff, which glows on being exposed to ultraviolet light, and which rapidly ceases to glow upon removal of ultraviolet light.

2. An oriented polyamide monofilament fishing line containing a fluorescent dyestuff which glows when exposed to ultraviolet light, which line has been stretched from $4\frac{1}{2}$ to 6 times its original length.

5. The fishing line of claim 2 in which the fluorescent dyestuff is present in from 0.05 to 0.5 wt. percent of the fishing line and is intimately mixed throughout the thickness of the said fishing line.

6. The fishing line of claim 5 in which the polyamide contains polymerized caprolactam.

8. The fishing line of claim 1 in which the dyestuff is distributed throughout the plastic.

[4] It is axiomatic that "one who appropriates the teachings of a patent may not deny the utility of the invention." *Tapco Products Co. v. Van Mark Products Corp.*, 446 F.2d 420, 428, 170 USPQ 550, 555-556 (6th Cir.), cert. denied, 404 U.S. 986, 172 USPQ 1 (1971); *Monogram Manufacturing Co. v. Glemby*, 136 F.2d 961, 963, 58 USPQ 443, 445-446 (2d Cir.), cert. denied, 320 U.S. 778, 59 USPQ 495 (1943); *Kansas City Southern Railway v. Silica Products Co.*, 48 F.2d 503, 505, 8 USPQ 476, 477-478 (8th Cir.), cert. denied, 284 U.S. 626 (1931). Hence, the first flaw was the failure to recognize that Berkley's admission, that its fishing line fell within the scope of claims 2, 5 and 6, is an admission of utility in the invention there claimed and that Berkley, as an infringer, was thereby estopped from asserting that those claims are invalid for lack of utility.¹¹

[5] The second flaw was the failure to recognize that the presumption of utility created by issuance of the patent, 35 U.S.C. §282, *Dashiell v. Grosvenor*, 162 U.S. 425, 432 (1896); *Strong-Scott Manufacturing Co. v. Weller*, 112 F.2d 389, 394, 45 USPQ 675, 679-680 (8th Cir. 1940); *Metropolitan Engineering Co. v. Coe*, 78 F.2d 199, 201, 25 USPQ 216, 217-218 (D.C. Cir. 1935);

¹¹ DuPont brought the fact of estoppel to the trial court's attention in a pretrial submission of proposed instructions. Berkley's briefs make no reference to the rule of law estopping an admitted infringer from asserting non-utility, or the rule of a strengthened presumption of utility when the invention is copied and becomes a commercial success.

Superior Hay Stacker Manufacturing Co. v. Dain Manufacturing Co., 208 F. 549, 557 (8th Cir. 1913), is strengthened when others, as Berkley did here, have copied the invention, *Superior Hay Stacker Manufacturing Co. v. Dain Manufacturing Co.*, id. at 557, and when, as it did here in both DuPont's and Berkley's hands, the invention has achieved commercial success, *Western Electric Co. v. LaRue*, 139 U.S. 601, 608 (1891); *Continental Can Co. v. Anchor Hocking Glass Corp.*, 362 F.2d 123, 124, 150 USPQ 1, 2 (7th Cir. 1966); *Panduit Corp. v. Stahl Bros. Fibre Works, Inc.*, 298 F.Supp. 435, 442, 162 USPQ 114, 121 (W.D. Mich. 1969), aff'd, 430 F.2d 221, 166 USPQ 524 (6th Cir. 1970), cert. denied, 401 U.S. 939, 168 USPQ 673 (1971).

Berkley, for the first time on appeal, says its non-utility position relates to the "ceases to glow" language of withdrawn claims 1 and 8.¹² The evidence respecting utility was insufficient, however, for any purpose.

The assertion of non-utility rests on the limited excitability of fluorescent dyes by the "visible" component of sunlight; hence, says Berkley, DuPont's line does not entirely cease to glow as long as visible light is present. Berkley then defines relative invisibility under water as less visibility than non-fluorescent line, and says DuPont's line fails that objective because sufficient visible light penetrates clear water to excite the fluorescent dye in the line.¹³

Berkley introduced photographs taken in clear water (the Crystal River and the Bermuda Triangle), and purportedly showing DuPont's line more visible than non-fluorescent lines at depths as great as 100 feet,¹⁴ along with narrated movies of similar scenes. Berkley's witness Lau said DuPont's

¹² Claims 1 and 8 were present below on a theory that their prosecution involved fraud on the PTO. The distinction from claims charged to have been infringed was not, however, made clear by the parties at trial.

¹³ Berkley does not challenge the patent's assertion that the fluorescent-dyed line is more visible above water, i.e., to the fisherman, than is transparent line. Though Berkley argues that DuPont must be held strictly to the language of the claims in relation to prior art, it relies on its interpretation of the specification for its argument that the invention fails to meet Keller's objectives and thus lacks utility.

¹⁴ DuPont challenges the evidence, pointing to the treatment of the fluorescent line during manufacture, giving it a milky appearance and making it more visible than the untreated "control" line, even when it is not fluorescing, as conceded in court by Berkley's patent expert.

line "glows all the way down to the bottom. * * * It glows like a light bulb." Roberts said the DuPont line was visible underwater.¹⁵

DuPont countered with the Johnson report, comparing the relative visibility of DuPont's fluorescent line and a similar but non-fluorescent nylon monofilament line in six different types of water, and with witnesses Shields and Cullerton, who said DuPont's line lost fluorescence inches below the surface of Lake McBride. Johnson's tests showed that the depth at which fluorescent and non-fluorescent lines appear identical varies from 7" for turbid seawater to more than 400" for distilled water. Dr. Johnson testified that his test results were consistent with the visibility language in the DuPont patent, which nowhere asserts that the submerged line is less visible than some other line. DuPont also introduced the McNally report, stating that DuPont's fluorescent line and a nonfluorescent line appeared identical at a depth of 5 feet in a lake "clearer than the average fishing lake."

[6] Non-utility of the invention is not established by the recitation in claims 1 and 8 that the dye "rapidly ceases to glow upon removal of ultraviolet light," when the claims are read, as they must be read, in their entirety.¹⁶ The claims and the patent nowhere mention visible light. Ultraviolet light is the sole glow-activating agent recited. One skilled in the art, reading the entire claim, and not charged with its infringement, would find the reasonable interpretation of the functional "ceases to glow" claim limitation to be that the glow produced by ultraviolet light rapidly ceases when that light is removed.

[7] Perfection under all conditions is not required,¹⁷ whether the patent does or does

not suggest that the invention is imperfect or inoperable under certain conditions. See *Conner v. Joris*, supra note 17, at 946-48, 113 USPQ at 57-60; *Plant Products Co. v. Charles Phillips Chemical Co.*, supra note 17 at 586, 37 USPQ at 652-653. Dr. Johnson's report showed the DuPont line no more visible than non-fluorescent line under some water conditions. Berkley did not challenge those test results, but relied on *ex parte* tests it conducted exclusively in crystal clear water. Assuming that Berkley's evidence establishes what it claims, i.e., that DuPont's line is more visible than non-fluorescent line in crystal clear water, that evidence is insufficient as a matter of law to establish non-utility. At most, it might demonstrate that DuPont's commercial embodiment of the invention does not work

beneficial function. *National Slug Rejectors, Inc. v. A.B.T. Manufacturing Corp.*, 164 F.2d 333, 335, 75 USPQ 151, 153-154 (7th Cir. 1947), cert. denied, 333 U.S. 832, 76 USPQ 621 (1948); *In re Oberweger*, supra at 828, 47 USPQ at 457-458; *Panduit Corp. v. Stahl Bros. Fibre Works, Inc.*, supra at 435, 162 USPQ at 114. An invention does not lack utility merely because the particular embodiment disclosed in the patent lacks perfection or performs crudely. *Hildreth v. Mastoras*, 257 U.S. 27, 34 (1921); *Decca Ltd. v. United States*, 544 F.2d 1070, 1077, 191 USPQ 439, 444-445 (Ct. Cl. 1976); *Field v. Knowles*, 183 F.2d 593, 600 (CCPA 1950); *Plant Products Co. v. Charles Phillips Chemical Co.*, 96 F.2d 585, 586, 37 USPQ 651, 652-653 (2d Cir. 1938); *Besser v. Merrilat Culvert Core Co.*, 243 F. 611, 612 (8th Cir. 1917). A commercially successful product is not required. *Hildreth v. Mastoras*, supra at 34; *In re Anthony*, 414 F.2d 1383, 1396, 162 USPQ 594, 605 (CCPA 1969). Nor is it essential that the invention accomplish all its intended functions, *Conner v. Joris*, 241 F.2d 944, 947, 113 USPQ 56, 58-59 (CCPA 1957); *Panduit Corp. v. Stahl Bros. Fibre Works, Inc.*, supra at 442, 162 USPQ at 121, or operate under all conditions, *Decca Ltd. v. United States*, supra at 1077, 191 USPQ at 444-445, partial success being sufficient to demonstrate patentable utility, *Freedman v. Overseas Scientific Corp.*, 248 F.2d 274, 276, 115 USPQ 42, 43-44 (2d Cir. 1957), *Emery Industries, Inc. v. Schumann*, 111 F.2d 209, 210, 45 USPQ 12, 12-13 (7th Cir. 1940), *Cummins Engine Co. v. General Motors Corp.*, 299 F.Supp. 59, 90, 161 USPQ 641, 658-659 (D.Md. 1969), aff'd, 424 F.2d 1368, 165 USPQ 618 (4th Cir. 1970). In short, the defense of non-utility cannot be sustained without proof of total incapacity. *Scovill Manufacturing Co. v. Satler*, 21 F.2d 630, 634 (D. Conn. 1927). Proof of inoperativeness or non-utility must be strong, *Steinfu Patents Corp. v. William Beyer, Inc.*, 62 F.2d 238, 240, 16 USPQ 219, 220-221 (2d Cir. 1932), every reasonable doubt being resolved in favor of the patentee, *Strong-Scott Manufacturing Co. v. Weller*, supra at 394, 45 USPQ at 679-680.

¹⁵ DuPont points to Lau's statement that Berkley lines made per the DuPont patent showed a "sharp visibility drop-off * * * at modest depths" and were "difficult to detect" at a depth of 15 feet in crystal clear water, and to Roberts' equivocal indication on redirect that "It stops glowing" and "I don't really think it stops glowing."

¹⁶ The claims measure the invention, *Graver Tank & Manufacturing Co. v. Linde Air Products Co.*, 336 U.S. 271, 277, 80 USPQ 451, 453 (1949); *Smith v. Snow*, 294 U.S. 1, 11, 24 USPQ 26, 30 (1934), but the invention must be viewed "as a whole," *Parker v. Flook*, 437 U.S. 584, 594, 198 USPQ 193, 199 (1978).

¹⁷ A small degree of utility is sufficient. *In re Oberweger*, 115 F.2d 826, 828, 47 USPQ 455, 457-458 (CCPA 1940). The claimed invention must only be capable of performing some

perfectly under a particular condition and is thus not a model of perfection.

It is instructive on the issue of utility that any imperfections respecting some continued glow under visible light in clear water did not deter Berkley from copying the invention and selling it for over 7½ million dollars during 1976-1978.¹⁸

[8] Thus, the issue of whether the patent was invalid for lack of utility should not have gone to the jury and should have been decided as a matter of law in DuPont's favor. Its submission, for any purpose, constituted prejudicial error.

C. Novelty

[9] A patent is invalid under 35 U.S.C. §§102(a)-102(b)¹⁹ if the claimed invention was "known or used by others" in this country prior to the patentee's invention or was "in public use or on sale" in this country more than one year before the application for the patent was filed. The burden of proof of a prior use is satisfied only by evidence clear, cogent, and convincing. *The Barbed Wire Patent*, 143 U.S. 275, 284 (1892); *Julian v. Drying Systems Co.*, 346 F.2d 336, 338, 145 USPQ 631, 632-633 (7th Cir. 1965); *McCullough Tool Co. v. Well Surveys, Inc.*, 343 F.2d 381, 394, 145 USPQ 6, 16 (10th Cir. 1965), cert. denied, 383 U.S. 933, 148 USPQ 772 (1966); *Atlas v. Eastern Air Lines, Inc.*, 311 F.2d 156, 160, 136 USPQ 4, 7 (1st Cir. 1962), cert. denied, 373 U.S. 904, 137 USPQ 912 (1963).

[10] Oral testimony alone has been held insufficient to prove a prior use. *Zachos v. Sherwin-Williams Co.*, 177 F.2d 762, 763, 83 USPQ 408, 408-409 (5th Cir. 1949). The more widespread view, however, is that unsupported oral testimony can be sufficient but must be regarded with suspicion and subjected to close scrutiny. *Miner v. T.H. Symington Co.*, 250 U.S. 383, 386 (1919); *Deering v. Winona Harvester Works*, 155

U.S. 286, 300 (1894); *Jones Knitting Corp. v. Morgan*, 361 F.2d 451, 455, 149 USPQ 659, 662-663 (3d Cir. 1966); *Becker v. Electric Service Supplies Co.*, 98 F.2d 366, 368, 38 USPQ 293, 294-295 (7th Cir. 1938); *Farmhand, Inc. v. Lahman Manufacturing Co.*, 192 USPQ 749, 755 (D. S.D. 1976), aff'd, 568 F.2d 112, 196 USPQ 597 (8th Cir.), cert. denied, 436 U.S. 913, 197 USPQ 848 (1978); See *The Barbed Wire Patent*, supra at 284-85; *Eibel Process Co. v. Minnesota & Ontario Paper Co.*, 261 U.S. 45, 60 (1923).²⁰

(1) The McCoy Line

In 1964, DuPont learned of a chartreuse fluorescent "Glo-Line" being sold by John McCoy in Seattle. McCoy told Greenwood, a DuPont salesman sent to investigate, that DuPont's 1949 records should contain inquiries from McCoy respecting fluorescent dyes useful in nylon. Greenwood found a sample of DuPont fluorescent dye, dated December 1949, in McCoy's possession. Greenwood's call reports reflect McCoy's allegations of having dyed nylon for over 15 years. McCoy said he had dyed yarn for lures since 1924. In a 1964 letter, McCoy said he had processed his Glo-Line since 1949. He said he made chartreuse fishing line "for the last two years or longer." In 1965, McCoy said he used the 1949 dye on lines for tying nymphs. In 1975, McCoy gave a written statement that he had first sold fluorescent fishing line in late 1964.

Five witnesses (3 live, 2 by deposition) said they had seen or sold McCoy's fluorescent chartreuse fishing line at least once in the mid-1950's. Kawahara said he sold it in

¹⁸ At oral argument, it was admitted that Berkley did not tell its customers that its copy of the invention lacked utility.

¹⁹ §102. Conditions for patentability; novelty and loss of right to patent.

A person shall be entitled to a patent unless —

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent, or

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of the application for patent in the United States

²⁰ Factors considered in determining the sufficiency of oral testimony are: (1) delay between event and trial, (2) interest of witness, (3) contradiction or impeachment, (4) corroboration (5) witnesses' familiarity with details of alleged prior structure, (6) improbability of prior use considering state of the art, (7) impact of the invention on the industry, and (8) relationship between witness and alleged prior user. See *Deering v. Winona Harvester Works*, supra at 300-01; *Jones Knitting Corp. v. Morgan*, supra at 456, 149 USPQ at 663-664; *A.J. Industries, Inc. v. Dayton Steel Foundry Co.*, 394 F.2d 357, 361, 157 USPQ 545, 548 (6th Cir. 1968); *Whiteman v. Mathews*, 216 F.2d 712, 716, 104 USPQ 83, 86 (9th Cir. 1954); *Becker v. Electric Service Co.*, supra at 368, 38 USPQ at 294-295; *Farmhand, Inc. v. Lahman Manufacturing Co.*, supra at 755; *A.B. Dick Co. v. Simplicator Corp.*, 34 F.2d 935, 939-40, 2 USPQ 428, 431-433 (2d Cir. 1929); *Eibel Process Co. v. Minnesota & Ontario Paper Co.*, supra at 60; *Smith v. Hall*, 301 U.S. 216, 222, 33 USPQ 249, 252 (1937).

his Seattle store before the 1958 introduction of DuPont's non-fluorescent line. Severeid said he saw McCoy's chartreuse line in Kawahara's store before transferring to his new office in August 1958. Sivertsen said he saw McCoy's chartreuse line before moving into his new store in 1957. Schalkle said he saw McCoy's chartreuse line before his transfer to a new job in 1954. Earling said he saw McCoy's chartreuse line about the time he was married in 1954.

Thus five witnesses claiming to have seen McCoy's chartreuse fishing line testified from memory to events of twenty years past. Though accompanied by reference to concurrent events, their testimony was inconsistent with the evidence produced by McCoy, the alleged prior user, whose one consistent indication was that his earliest claimed use of chartreuse fishing line was after Keller's invention, and who in 1964 possessed no chartreuse dye manufactured prior to 1963. Severeid, Sivertsen, Schalkle and Earling had no corroborating documentary or physical evidence. All five witnesses said they saw only McCoy's chartreuse line, and though all said they saw it between 1954 and 1958, Berkley declined to review McCoy's available and offered sales records for 1952 through 1958. McCoy identified a 1964 invoice (two years after the DuPont patent issued) as representing one of his first sales of fluorescent line. McCoy's 1954 ad did not mention fluorescent fishing line, and his 1949 dye sample does not evidence use of fluorescent dye in a fishing line. The only physical evidence produced was a spool of line Kawahara said was one of the first he had purchased, and DuPont's unchallenged tests proved that line had not been made prior to 1962.²¹

[11] Berkley thus failed as a matter of law to carry its burden of proving prior use by McCoy. The attempt rests solely on uncorroborated oral testimony concerning events of long ago, contradicted by McCoy himself and by unchallenged results of scientific tests. Judged by appropriate legal standards, that testimony had insufficient probative value to make out a case of prior use that a jury could have found clear and convincing. Thus prejudicial error occurred in the instruction to the jury that it could find the DuPont patent invalid as anticipated by the McCoy line.

²¹ Berkley says it learned of DuPont's spool tests only a few days before the end of trial, but does not charge the trial court with an abuse of discretion in admitting the testimony.

(2) *The Cohantic Line*

A spool of "Cohantic" line was allegedly discovered by Berkley in a fishing line "morgue" of the Cortland Line Company in New York. Placed under a strong artificial ultraviolet light, the line gave off a faint glow, about 3% of that of DuPont's patented line. The glow was a little more in portions of the spool that had been covered for years by a rubber band and thus protected from light and oxidation. The Cohantic line did not glow in sunlight.

Berkley's expert Stearns compared the glow of the Cohantic line to the inherent glow of a piece of undyed, relatively stiff, fishing leader of unknown composition. Finding a difference in spectra and intensity, Stearns concluded that the glow in the Cohantic line was caused by the presence of a "brightener," by which he apparently meant a fluorescent dye.

DuPont's expert Jenkins found that the glow spectra of the Cohantic line and the inherent glow of undyed line made of the same nylon were substantially identical.

DuPont's request that we find fluorescent dye absent from the Cohantic line, and Berkley's request that we find it present, are inappropriate at this stage.²² The testimony is equivocal. Neither side conducted scientific tests for the presence of fluorescent dye, though DuPont says the amount, if any, would be too minuscule for scientific testing. That Stearns' comparison line was not of the same nylon, while Jenkins' was, that many materials (assertedly including fingernails), inherently fluoresce under strong artificial ultraviolet light, and that a protected line portion glowed more than an unprotected portion, were matters for the jury, in its evaluation of the credibility and reliability of the witnesses and their testimony.

The trial court correctly submitted the evidence of the Cohantic line to the jury. The absence of indication that the jury found the patent invalid because of the Cohantic line, the gaps and conflicts in the Cohantic line evidence, and the prejudicial errors in submission of the non-utility and McCoy line matters, all preclude a sustaining here of the jury's invalidity verdict on the basis of the Cohantic line evidence.

²² Claim 5 specifies a particular quantity of dye. There was no evidence concerning any specific amount of dye in the Cohantic line. We indicate no view respecting whether the Cohantic line constitutes a prior use of Keller's invention.

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D. Obviousness

[12] As this court noted in *Woodstream Corp. v. Herter's, Inc.*, 446 F.2d 1143, 1149, 170 USPQ 380, 384 (8th Cir. 1971), application of 35 U.S.C. §103²³, i.e., determination of the obviousness issue, is governed by the principles articulated by the Supreme Court in *Graham v. John Deere Co.*, 383 U.S. 1, 17-18, 148 USPQ 459, 467 (1966):

While the ultimate question of patent validity is one of law, *A. & P. Tea Co. v. Supermarket Equipment Corp.*, [340 U.S. 147, 155, 87 USPQ 303, 307 (1950)] the §103 condition, which is but one of three conditions, each of which must be satisfied, lends itself to several basic factual inquiries. Under §103, the scope and content of the prior art are to be determined; differences between the prior art and the claims at issue are to be ascertained; and the level of ordinary skill in the pertinent art resolved. Against this background, the obviousness or nonobviousness of the subject matter is determined. Such secondary considerations as commercial success, long felt but unsolved needs, failure of others, etc., might be utilized to give light to the circumstances surrounding the origin of the subject matter sought to be patented. As indicia of obviousness or nonobviousness, these inquiries may have relevancy.

[13] Though the ultimate question of obviousness is subject to evaluation on appeal, *Flour City Architectural Met. v. Alpina Aluminum Products, Inc.*, 454 F.2d 98, 106, 172 USPQ 341, 347-348 (8th Cir. 1972); see *Sakraida v. Ag Pro, Inc.*, 425 U.S. 273, 280, 189 USPQ 449, 452 (1976); *Graham v. John Deere Co.*, supra at 17, 148 USPQ at 466-467, we decline an independent evaluation on the present record. In the ordinary case, the appellate court has the benefit of the factfinder's conclusion that the invention would or would not have been obvious, and

²³ §103. Conditions for patentability; non-obvious subject matter.

A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

the facts and reasons underlying the conclusion. Here the jury announced no conclusion, either way. There are no findings of fact, and the trial court said it was making no determination of the question.

Even in non-jury cases, where the trial court made no findings or conclusions on obviousness, appellate courts have declined the initial determination of that question. As was said in *Cloud v. Standard Packaging Corp.*, 376 F.2d 384, 391, 153 USPQ 317, 322-323 (7th Cir. 1966):

But, in general, and we think in this case as well, it is sounder procedure for the initial determination to be made in the trial court. That court has advantages not only in determining credibility of those it sees and hears, but it has much greater flexibility, where it deems it desirable, to call counsel before it for colloquy, or to order supplementation of the evidence. And where the determination of this type of issue is first made in the court of appeals, there is no court where the parties can obtain review as a matter of right.

We refrain, therefore, from attempting to decide the matter at this stage, and direct further proceedings in the district court to determine the challenge to all these patents for obviousness under 35 U.S.C.A. §103. [Footnote omitted.]

See also *Kockum Industries, Inc. v. Salem Equipment, Inc.*, 467 F.2d 61, 64, 175 USPQ 81, 83 (9th Cir. 1972), cert. denied, 411 U.S. 964, 177 USPQ 545 (1973); *Sutherland Paper Co. v. Grant Paper Box Co.*, 183 F.2d 926, 935-36, 86 USPQ 337, 344-345 (3d Cir.), cert. denied, 340 U.S. 906, 87 USPQ 432 (1950).

[14] As the Supreme Court said in *Graham*, the obviousness-nonobviousness conclusion rests on factual inquiries, which were to have been resolved in the first instance in this case by a jury. The record reflects extensive conflicts in testimony, and no indication as to how, if at all, the jury resolved those conflicts. These considerations, coupled with errors which appear to have deprived the jury of a true picture of the issue, dictate the avoidance of potential injustice inhering in an attempted resolution of the required factual inquiries on the basis of the cold paper record before us.

[15] Having resolved the factual inquiries necessitated by the evidence presented to it, the jury at retrial must, if so requested, then reach a final conclusion respecting ob-

viousness.²⁴ Avoidance of subjectivity and the insidious effect of hindsight, i.e., resisting "the temptation to read into the prior art" the teachings of Keller's invention, Graham, *supra* at 36, 148 USPQ at 474, may prove difficult. Avoiding those analytical defects, the jury may conclude, on the basis of all the evidence, that it would or would not have been obvious to have made Keller's invention in 1961. If that conclusion be accompanied by answers to interrogatories or special verdicts indicating its factual underpinnings, an appeal on the issue may be rendered either unnecessary or more easily managed.

[16] The parties are entitled to a consideration in the first instance of all the facts touching the obviousness issue, by a factfinder who sees and hears the witnesses, and to that consideration free of the errors that occurred below.²⁵

(1) The French Line Error

A fishing line manufactured and sold in France in the 1950's and allegedly identical to DuPont's invention was allowed into evidence. DuPont challenged its admissibility, arguing that the jury would erroneously treat it as evidence of prior art.

If the French line is the same invention as Keller's,²⁶ it was admitted here under erroneous instructions.

[17] A prior use or sale constitutes "prior art" only if it occurs "in this country." 35 U.S.C. §102. Hence prior use in a foreign

country is not prior art for the purpose of determining obviousness under section 103, and evidence respecting the French line was inadmissible to prove prior art, the line having never been used or sold in this country.

[18] In admitting the evidence, the court instructed the jury to consider it "solely as it bears on the *state of the art* and as it relates to the ultimate question of obviousness." (emphasis ours). That instruction supplied no guidance from which the jury could be expected to have distinguished the French line from the prior art. The court's instruction, considered as a whole, was thus misleading and erroneous.²⁷

Berkley's brief mistakenly tells us that the court phrased its instruction in terms of "the skill in the art."²⁸ The jury, however, would have been no less misled had that been true. The distinction is fine between "prior art" and either "state of the art" or "skill in the art". The possibility of a jury confusing evidence admitted to show either of the latter with other evidence admitted to show "prior art" weighs heavily against use of a cautionary instruction phrased in the terms here employed.

[19, 20] Evidence demonstrating contemporaneous foreign invention by another has been considered even though it does not

²⁷ Confusion was not limited to the jury box:

Mr. Tramontine [Counsel for DuPont]: Your honor, as I understand the use of the term "state of the art" by counsel [for Berkley], he means equivalent to prior art under Section 103.

The Court: Right.

The reference to obviousness in the instruction compounds the error. Preceded by "and", it at best tells the jury it may choose to consider the French line as evidence of the state of the art as distinguished from evidence of obviousness.

²⁸ The trial court referred elsewhere to counsel's statement that the evidence showed the level of skill in the art. Berkley cites one case in which a court said contemporaneous invention is a gauge of the level of skill in the art, *Standun, Inc. v. Polycraft Corp.*, 426 F.Supp. 649, 655-56, 191 USPQ 710, 715-716 (N.D. Ill. 1976), *aff'd*, 550 F.2d 395 (7th Cir. 1977). That was, however, a non-jury trial in which the court said it was using evidence of contemporaneous invention merely to "bolster" its earlier conclusion, based solely on the prior art. Berkley's other citation, *Stamicarbon, N.V. v. Escambia Chemical Corp.*, 430 F.2d 920, 166 USPQ 362 (5th Cir.), *cert. denied*, 400 U.S. 944, 167 USPQ 705 (1970), was also a non-jury trial, in which the court admitted evidence of foreign contemporary invention, but found the patent *valid* nonetheless, and stated (at 928) that the evidence "might not be admissible to prove prior art under 35 U.S.C. §102."

²⁴ Under Rule 402, Fed. R. Evid., all relevant evidence is admissible (unless forbidden by the Constitution, statute, or rule). It would, of course, be unjust, unfair, and unjudicial to ignore any unforbidden evidence relevant to the obviousness-nonobviousness question.

²⁵ Though vigorously argued by DuPont, we find no reversible abuse of discretion in admission of the testimony of Berkley's patent law expert. If a patent law expert testifies at retrial, however, care should be taken to avoid the close skating toward usurpation of court and jury functions illustrated, for example, by:

[Berkley's expert] Maybe I can satisfy both objections by saying if you find this fishing line to have been used or sold in the 1950's, based on the evidence, then it constitutes prior public use to the extent that it discloses a fishing line which has a fluorescence to it, and meets the terms of the Keller claims. It certainly — If it is prior art — and that is certainly for the jury — the question is what its application is, and it certainly would be highly relevant prior art.

²⁶ Two French lines were introduced. Only one was apparently alleged to be the same as Keller's invention, an allegation denied by DuPont.

qualify as prior art. *Stamicarbon, N.V. v. Escambia Chemical Corp.*, supra note 28; *Reeves Bros., Inc. v. U.S. Laminating Corp.*, 417 F.2d 869, 872, 163 USPQ 577, 579 (2d Cir. 1969). The similarity between contemporaneous invention and prior art dictates, however, that evidence of the former be cautiously admitted in a jury trial. The trial judge must carefully instruct the jury that the evidence is merely one possible indicium of obviousness. See *Reeves Bros., Inc. v. U.S. Laminating Corp.*, id., at 872, 163 USPQ at 579. That the same invention was contemporaneously made elsewhere may or may not, in the light of all the circumstances, be some indication that the invention would have been obvious, as the statute requires, to "one of ordinary skill in the art." Nothing should be more clear in the law of patents than the concept that the same patentable invention may be contemporaneously made by more than one inventor.²⁹

(2) The Presumption of Validity Error

Because the French and Cuculo patents were classified in files searched by the examiner, DuPont sought this instruction:

It is also assumed that the examiner reviewed the prior art which was in the files which he searched during the prosecution of the application for the patent.

Berkley objected, and the court omitted the requested statement.

[21] The statutory presumption of validity³⁰ flows from a congressional

assumption that the PTO properly performs its administrative functions. *Morgan v. Daniels*, 153 U.S. 120, 124-25 (1894); see *Chicago Rawhide Manufacturing Co. v. Crane Packing Co.*, 523 F.2d 452, 458, 187 USPQ 540, 545-546 (7th Cir. 1975), cert. denied, 423 U.S. 1091, 188 USPQ 480 (1976); *Beckman Instruments, Inc. v. Chemtronics, Inc.*, 439 F.2d 1369, 1374, 165 USPQ 355, 359-360 (5th Cir.), cert. denied, 400 U.S. 956, 168 USPQ 1 (1970); *Neff Instruments Corp. v. Cohu Electronics, Inc.*, 298 F.2d 82, 86, 132 USPQ 98, 100-101 (9th Cir. 1961). The presumption is not conclusive and can be rebutted by proof that the PTO erred. That a reference was cited or assumedly considered does not preclude a court or jury from being convinced that the patent should have been refused in view of that reference. That is not to say, however, that the presumption of validity is limited to those references actually cited by the examiner.

[22] The PTO's function entails a thorough scrutiny of prior art references.³¹

Under 35 U.S.C. §282 (1976), a patent is presumed valid and the burden of establishing invalidity rests on the party asserting it. *Blonder-Tongue Laboratories, Inc. v. University of Illinois Foundation*, 402 U.S. 313, 335, 169 USPQ 513, 522 (1971); *Woodstream Corp. v. Herter's, Inc.*, supra at 1149, 170 USPQ at 384. The mandate of section 282 is twofold, requiring "that a party asserting invalidity bear not only the presumption-generated burden of going forward with proof but also the burden of persuasion on that issue." *Solder Removal Co. v. International Trade Commission*, 582 F.2d 628, 632-33 n.8, 199 USPQ 129, 132-133 n.8 (CCPA 1978). The latter burden remains upon the party asserting invalidity whether relevant prior art was or was not considered by the examiner during prosecution of the patent application before the PTO. *Solder Removal Co. v. International Trade Commission*, id. at 632-33, 199 USPQ at 132-133; *Woodstream Corp. v. Herter's, Inc.*, supra at 1156, 170 USPQ at 389-390; See *Champion Spark Plug Co. v. The Gyromat Corp.*, 603 F.2d 361, 366-67, 202 USPQ 785, 788-789 (2d Cir. 1979). The presumption is fully rebutted only when the party asserting invalidity meets the burden of persuasion, i.e., relies on evidence that does in truth render the claimed invention invalid, though rebuttal may be easier when the prior art relied on is more relevant than that considered by the examiner. *Solder Removal Co. v. International Trade Commission*, supra at 632-33, 199 USPQ at 132-133; *Woodstream Corp. v. Herter's, Inc.*, supra at 1156, 170 USPQ at 389-390.

³¹ 35 U.S.C. §131 (1976), provides:

§131. Examination of application

The Commissioner shall cause an examination to be made of the application and the al-

²⁹ The statute establishing interferences in the PTO, 35 U.S.C. §135, is entirely premised on the concept that the same nonobvious invention may be contemporaneously made by a plurality of inventors. That congressional mandate, and the priority status given in interferences to the first-to-file a patent application, accord with the Constitutional purpose of the patent system, i.e., to encourage public disclosure of inventions in this country.

³⁰ 35 U.S.C. §282 (1976) provides, in relevant part:

§282. Presumption of validity; defenses

A patent shall be presumed valid. Each claim of a patent (whether in independent, dependent, or multiple dependent form) shall be presumed valid independently of the validity of other claims; dependent or multiple dependent claims shall be presumed valid even though dependent upon an invalid claim. The burden of establishing invalidity of a patent or any claim thereof shall rest on the party asserting such invalidity.

The PTO Rules of Practice require the examiner to cite only what he considers the "best references."³² The "file wrapper," i.e., the prosecution history, of a patent application lists the references cited against the application and the classes and subclasses of references inspected by the examiner.³³ Several courts have held that the examiner's search record is prima facie evidence that he considered all the references classified in the classes and subclasses searched and that he left uncited those he regarded as less relevant than those cited. *Panduit Corp. v.*

leged new invention; and if on such examination it appears that the applicant is entitled to a patent under the law, the Commissioner shall issue a patent therefor.

37 C.F.R. §1.104(a) (1978) provides:
§1.104. Nature of examination; examiner's action.

(a) On taking up an application for examination, the examiner shall make a thorough investigation of the available prior art relating to the subject matter of the invention sought to be patented. The examination shall be complete with respect both to compliance of the application with the statutes and rules and to the patentability of the invention as claimed, as well as with respect to matters of form, unless otherwise indicated.

³² 37 C.F.R. §1.106(b) (1978) provides:
§1.106 Rejection of claims.

(b) In rejecting claims for want of novelty or for obviousness, the examiner must cite the best references at his command. When a reference is complex or shows or describes inventions other than that claimed by the applicant, the particular part relied on must be designated as nearly as practicable. The pertinence of each reference, if not apparent, must be clearly explained and each rejected claim specified.

M.P.E.P. §707.05 (1978) provides in relevant part:

707.05 Citations of References

During the examination of an application the examiner should cite appropriate prior art which is nearest to the subject matter defined in the claims. When such prior art is cited, its pertinence should be explained.

³³ M.P.E.P. §904.01(d) (1978) provides, in relevant part:

904.01(d) Outlining Field of Search

An examiner, in each first action upon an application, makes an initialed endorsement in ink in the space provided on the left-hand page of the open file wrapper, stating the classes and subclasses of domestic and foreign patents, abstract collections and the publications in which search for references was made and also the date of the search.

Burndy Corp., 517 F.2d 535, 538 n.2, 186 USPQ 75, 77-78 n.2 (7th Cir.), cert. denied, 423 U.S. 987, 188 USPQ 48 (1975); *Elgen Manufacturing Corp. v. Grant Wilson, Inc.*, 285 F.2d 476, 479, 128 USPQ 168, 170-171 (7th Cir. 1961); *Farmhand, Inc. v. Lahman Manufacturing Co.*, *supra*, at 760. A contrary view would destroy the presumption of administrative regularity on which the presumption of validity rests.

[23] Assuming, *arguendo*, that Berkley's expert is correct in his generalized assertion at trial that references may occasionally be missing from the files, Congress, in enacting the presumption of validity, chose to assume prima facie that an oversight of relevant prior art did not occur. In view of the hundreds of patents in a single class or subclass, a requirement that the examiner cite every patent inspected would unreasonably retard the examination process. Thus, absent contrary evidence, it is improper to conclude that references not specifically cited by the examiner, but classified in areas he searched, were not considered by him. Cf. *Hobbs v. United States*, 451 F.2d 849, 863-64, 171 USPQ 713, 723-724 (5th Cir. 1971).

[24] The trial court's instruction that the "presumption does not extend or exist as to prior art patents or publications which do not appear from the record of the file wrapper," and the refusal to give DuPont's requested instruction, left the jury unapprised of the full extent of the statutory presumption of validity.³⁴

II. Fraud and Antitrust

The crux of Berkley's antitrust counterclaim was that DuPont knew, or in the exercise of due care should have known, of information that rendered its invention unpatentable but chose to fraudulently conceal that information from the examiner during prosecution of its patent application and further chose to ignore even more such information and enforce the patent, all in a scheme to maintain a monopoly in the fluorescent monofilament fishing line market in violation of Section 2 of the Sherman Act.³⁵

³⁴ Though instructions must be considered in their entirety, the court's reference to patents that "had not been considered . . . if they are more pertinent than those considered," as part of a single sentence with the statement quoted in the text, could not cure the potential for misdirection of the jury.

³⁵ Section 2 of the Sherman Act, 15 U.S.C. §2 (1976), provides:

§2. Monopolizing trade a felony; penalty

Because the jury returned its verdict for DuPont on Berkley's allegations of fraud and patent enforcement, the trial court immediately dismissed the counterclaim, finding the second part of the trial unnecessary.

On appeal, Berkley asks us to adopt its version of parts of the evidence. Having found the jury's verdict fully supported by the weight of all the evidence, and Berkley's versions unfounded, we decline that invitation. Berkley also charges the commission of errors allegedly necessitating reversal of the verdict or a new trial. We treat each issue, setting out the facts as they pertain to each.³⁶

A. Gross Negligence³⁷

Accusing DuPont of "gross negligence" in its investigation of facts allegedly placing it on notice that one or more claims of its pa-

Every person who shall monopolize, or attempt to monopolize, or combine or conspire with any other person or persons, to monopolize any part of the trade or commerce among the several States, or with foreign nations, shall be deemed guilty of a felony, and, on conviction thereof, shall be punished by fine not exceeding one million dollars if a corporation, or, if any other person, one hundred thousand dollars, or by imprisonment not exceeding three years, or by both said punishments, in the discretion of the court.

³⁶ The jury's findings on fraud and enforcement being supported by the evidence, we need not discuss the conflicting interpretations, charges of factual misstatements, and the requested inferences of intent, with which the parties have presented to us the absent (later delivered) attorneys' opinion in exhibit D-7 and the Payne presentation of the Johnson report.

³⁷ Because the evidence could not support a finding of gross negligence, we need not and do not decide whether gross negligence may be adequate basis for a charge of antitrust violation. The cases cited by Berkley thrust in a contrary direction or conflict with this court's view as expressed in *Agrashell, Inc. v. Hammons Products Company*, 479 F.2d 269, 287, 177 USPQ 501, 512-513 (8th Cir. 1973). In a post-argument submission, Berkley cites *W.R. Grace & Co. v. Western U.S. Industries, Inc.*, No. 75-2574, 75-2513, 203 USPQ 721 (9th Cir., Oct. 9, 1979), but that case involved fraud, and the court held the corporation liable, against its employee's denial of actual knowledge of falsity in his representation to the PTO. It is sufficient here that we agree with the trial court, in its ruling on post trial motions, that there was simply no basis in the "evidence in this case" for submitting to the jury any issue of DuPont's failure to inquire into validity of its patent.

DuPont says Berkley did not plead gross negligence. But Berkley pled "fraudulent maintenance," and ambiguous pleadings are to be construed liberally in favor of the pleader.

tent might be invalid, Berkley charges error in the trial court's refusal to instruct and submit to the jury an interrogatory respecting DuPont's gross negligence prior to issuance and enforcement of its patent. The accusation rests on DuPont's conduct respecting the McCoy line, the Starlite line, and reports on underwater visibility of DuPont's fluorescent line product.

In 1964, two years after its patent issued, when DuPont learned of McCoy's Glo-line product, its investigation of the McCoy story was immediate and thorough. Resulting information concerning the nature and timing of McCoy's use of fluorescent dye was decidedly vague and indefinite. McCoy's statements were inconsistent. No physical or documentary evidence establishing McCoy as a prior user of fluorescent monofilament fishing line ever appeared.³⁸ Every statement of McCoy about his chartreuse fishing line placed his first use after Keller's invention. McCoy's decision, made after the patent issued, to abandon his fluorescent line was inconsis-

Hanson v. Hunt Oil Co., 398 F.2d 578, 581 (8th Cir. 1968). Berkley cites dicta in *Norton v. Curtiss*, 433 F.2d 779, 794-96, 167 USPQ 532, 544-546 (CCPA 1970), indicating that a patent application may be struck by the PTO if a misrepresentation is made to it with knowledge of falsity or in an "atmosphere" of gross negligence as to truth.

³⁸ Paul Johnson of Berkley visited McCoy in December of 1975 and gave the following testimony at trial about his visit:

We asked if he had any invoices or sales records, and he said that he didn't have them anymore; that, after duPont people visited him [in September of 1975], they threw them all out. They didn't exist.

At his deposition, taken by DuPont in July of 1977, McCoy, who was 79 by then, produced his invoices for the years 1954-1958 and after 1964. He no longer had invoices for the years 1959-1963. McCoy's explanation for the missing 1959-1963 invoices was:

Mr. Churchill [counsel for DuPont]: What I am getting at, Mr. McCoy, is that some of these records for these years are missing.

Mr. McCoy: Oh, yes.

Mr. Churchill: What happened to them, can you tell us?

Mr. McCoy: I don't know. I don't know what happened to them.

Mr. Churchill: Were they destroyed in some way?

Mr. McCoy: They may have been. They could have been.

DuPont asserts that those same 1959-1963 invoices were missing when its representatives visited McCoy in September of 1975.

tent with his claim of prior use, notwithstanding his desire to be "friends" with DuPont and Berkley's inference that he must have been "pressured." Keller concluded that "McCoy was just another person who was claiming to have had a fluorescent fishing line prior to my invention, and yet had no evidence to back it up." In September, 1975, McCoy gave DuPont's representatives a December, 1964, invoice and a signed statement that it represented one of his first sales of fluorescent line.

Under all the circumstances, DuPont was not guilty of gross negligence in concluding that McCoy's original vague and inconsistent implication of a prior use was groundless. To require patentees to do more than was here done, when faced with such unsubstantiated allegations, would put them at the mercy of every crank and charlatan who suddenly "remembers" a prior use of the patentee's invention after the patent is issued.

In 1962, Sunset, the company manufacturing Starlite line, was a sales agent for DuPont fishing line. When DuPont's patent issued, a copy went to all sales agents. Sunset received its copy on December 14, 1962, and on December 18, its president Agnew wrote DuPont:

For your information we put illuminous powders in plastic four or five years ago and when we shine a black light on them, they actually made the line look like it was completely purple.

On January 4, 1963, Tyner of DuPont thought the Starlite line might affect validity of the DuPont patent. Consequently, in early 1963, Keller was sent to investigate Agnew's assertion. Agnew said the Starlite line was a phosphorescent fly line, charged up with a flashlight and used at night, to glow continuously for 10 or 15 minutes after the light was removed, above and under water.

Though DuPont might have tested the Starlite line for the presence or absence of fluorescent dye,³⁹ its investigation was more than sufficient to avoid a charge of gross negligence. It promptly investigated Agnew's assertion and could not be held grossly negligent for relying upon what

Agnew told and showed Keller. Berkley's expert Stearns admitted that one looking at the Starlite line would be unable to distinguish any fluorescence in it from the phosphorescence. Keller's conclusion that Agnew's phosphorescent fly line had no relevance to DuPont's fluorescent fishing line was not grossly negligent.

[25] At DuPont's direction, Dr. Johnson had completed his tests and a report on underwater visibility of DuPont's fluorescent line on March 14, 1962, several months before issuance of the DuPont patent. Johnson tested the line under a wide variety of conditions and concluded that it worked as described in the patent application and was useful. Berkley's expert Stearns said Johnson had prepared "a very good scientific reliability report" of which "any scientific lab would be proud," and that Dr. Johnson's tests were all that was necessary to determine how the fishing line would work.⁴⁰

The report's suggestion that visibility of the line bore an inverse relationship to clarity of the water does not contradict or otherwise invalidate Johnson's conclusion. Nor was his conclusion contradicted by reports of Roberts and Lau to DuPont before suit. Roberts said only that the line did not completely disappear underwater and Lau's statement was based solely on observations made in crystal clear water and thus was not inconsistent with Johnson's report. Johnson's conclusions were corroborated by McNally, a scuba diver hired by DuPont to make underwater observations of its line.

In view of the extensive testing by DuPont, its tests having confirmed the presence of patentable utility, it cannot be said that DuPont was grossly negligent in determining whether its invention worked.

Citing no specific conduct by DuPont, Berkley adds this vague allegation:

No one knows which pieces of prior art formed the basis for the jury's determination of invalidity. However, it is very possible that the jury determined that the Keller patent was invalid on the basis of prior art which was admittedly known to DuPont prior to the issuance or enforce-

³⁹ No such test was ever reported by either party. Stearns testified that he found fluorescence in ex parte laboratory tests but did not identify the source of that fluorescence.

Agnew and Keller dispute the fact of a 1962 conversation. If it occurred, DuPont's failure to act on it did not constitute gross negligence.

⁴⁰ Stearns also said the report proved Keller's invention "did not work" because it showed the line was not invisible against a dark background or the sky. As discussed *supra* invisibility was not required for utility. Moreover, that Berkley's expert took a particular view of the Johnson report at trial does not establish gross negligence in failure of DuPont to take the same view before or after trial.

ment of the patent. If the jury concluded, on the basis of such facts, that the Keller patent was invalid, the jury may very well have concluded that duPont was grossly negligent in failing to make a similar determination prior to the issuance or enforcement of the patent.

[26] The notion that DuPont could be found guilty of gross negligence for failure to anticipate a jury's verdict is meritless. Moreover, the jury's verdict may have rested on non-utility, with no basis in the prior art. None of the prior art or other information known to DuPont, in any event, so plainly rendered its invention unpatentable that DuPont could be held guilty of gross negligence in accepting its patent and exercising its right to seek legal enforcement in the courts.

There being no evidence warranting submission of a gross negligence issue to the jury, the trial court committed no error in refusing to give Berkley's requested instruction and interrogatory.

B. Jury Instructions on Withholding the Johnson Report

Focusing exclusively upon one paragraph of an 8 paragraph instruction (No. 21), Berkley maintains that the court erroneously permitted the jury to find fraud only if DuPont willfully withheld "prior art" from the PTO. The challenged paragraph describes Berkley's burden of proof and speaks only of withholding "prior art." Thus, says Berkley, if the jury found that DuPont withheld scientific data, i.e., the Johnson report, but had not withheld "prior art," it could have erred in finding DuPont not guilty of fraud.

Jury instructions must be viewed in their entirety and verdicts will not be overturned by picking and choosing words from an instruction without regard to the realities of the trial. *Fields v. Chicago Rock Island and Pacific Railroad*, 532 F.2d 1211, 1213-14 (8th Cir. 1976); *Jiffy Markets, Inc. v. Vogel*, 340 F.2d 495, 500 (8th Cir. 1965).

Instruction No. 21, read as a whole, emphasizes the requirement for "[a]bsolute honesty and good faith disclosure," the prohibition of "suppression of pertinent facts," and the "rule of absolute candor with the Patent Office." It states that an "omission of material facts" may constitute "fraud" rendering a "patent invalid even if the fraud pertains to only one claim." Thus the instruction as a whole clearly conveys the concept that the withholding of any relevant material, described in the instruction

as "prior art," "facts," and "information," may form the basis for a finding of fraud. Berkley at trial stressed the withholding of the Johnson report. There was no real possibility that the jury was misled into believing that it could not consider the withholding of the Johnson report in reaching its decision on fraud.⁴¹

C. Interrogatories 2 and 3⁴²

Berkley contends that the court erred in phrasing Special Interrogatory 2 as "Did plaintiff obtain the * * * [DuPont] patent from the Patent Office by fraud?" and Special Interrogatory 3 as "Did plaintiff assert its patent against Berkley knowing that it was invalid?"

Berkley sought interrogatories asking whether DuPont had obtained by fraud or enforced, knowing to be invalid, "any one or more claims," arguing that fraudulent conduct respecting one claim renders the entire patent invalid, and that enforcement of one

⁴¹ Berkley's primary position on fraud is that, because the Johnson report showed DuPont's line more visible than non-fluorescent line under some water conditions, it would have rendered DuPont's patent invalid for lack of utility and its withholding thus constituted fraud. As indicated in the text, *supra*, the Johnson report would not have demonstrated absence of utility. Nor does the file wrapper indicate that the PTO issued the patent in the belief that the addition of fluorescent dye rendered the line totally invisible or less visible than another line at all depths and in all conditions of water. Nonetheless, the jury's no-fraud finding may have been based on a conclusion that, though Keller's invention was shown at trial as lacking in utility, DuPont was not chargeable with knowing and concealing that fact.

Berkley asserts that DuPont failed to disclose to the PTO its dye booklet and its experiments with dye in nylon powders. We are not told, nor do we see, how those matters are more pertinent than the 16 items disclosed by DuPont to the PTO and the 8 patents cited by the examiner.

⁴² In its Statement of the Issues Presented for Review, Berkley did not request review of the jury's answer to Interrogatories 2 and 3. Nor did it argue that issue. In the conclusion of its initial brief, however, Berkley alleged broadly that the answers were "contrary to the great weight of the evidence." When DuPont asserted waiver of the issue, Berkley reiterated the same broad attack, adding that it "has not abandoned the issue" and that "its entire brief deals with the issue." Berkley's briefs reveal little more than catalog descriptions of information allegedly known to DuPont, followed by broad assertions that the examiner was never advised of the information. No direct evidence that DuPont acted in bad faith is cited. It is unnecessary to decide whether Berkley waived the issue. The evidence adduced at trial was more than ample to support the jury's answers to Interrogatories 2 and 3.

claim knowing it to be invalid renders the patentee liable under the antitrust laws even if the remaining claims asserted are valid.⁴³

The submission and form of interrogatories to the jury are matters within the sound discretion of the trial court, and review is confined to a determination of whether there was an abuse of discretion. *Tights, Inc. v. Acme-McCrary Corp.*, 541 F.2d 1047, 1060 (4th Cir.), cert. denied, 429 U.S. 980 (1976); *Dreiling v. General Electric Co.*, 511 F.2d 768, 774 (5th Cir. 1975); *McDonnell v. Timmerman*, 269 F.2d 54, 58 (8th Cir. 1959).

Jury Instruction No. 21 included:

[A] fraud has been perpetrated on the Patent Office and the patent is rendered invalid even if the fraud pertains to only one claim.

Being so instructed, the jury, in giving its "no" answer to Interrogatory No. 2, had before it the concept that DuPont had to have procured every claim of its patent free of fraud. The contested issue having thus been adequately presented to the jury, it cannot be said that the court abused its discretion in phrasing Interrogatory 2.⁴⁴

Concerning Interrogatory No. 3, Berkley speculates that:

The evidence in this case disclosed test results and numerous pieces of prior art, of which duPont had knowledge, that *may* have invalidated one or more, but not all, of the claims of the Keller patent

[A] careful examination of each piece of prior art and each test result introduced into evidence at trial *could arguably* have been construed by the jury to invalidate one or more claims of the Keller patent, without invalidating all of the claims of the patent. Additionally, a jury *could have* concluded that even though a piece of prior art invalidated all of the claims of the Keller patent, duPont's ac-

tual knowledge was such that duPont was only aware that certain claims of said patent were invalid when it decided to file suit against Berkley. [Emphasis added.]

Berkley was ultimately charged with infringement of only claims 2, 5 and 6. It has offered not even speculation as to how the jury might have concluded that DuPont enforced one or two but not all three of those claims believing them to be invalid. Though DuPont initially asserted claims 1 and 8, there is no evidence from which a jury could find that DuPont "knew" those claims were invalid when it filed its complaint. Because no charge of enforcement of any claim believing it to be invalid could properly lie, the interrogatory sought by Berkley could only have been prejudicial to DuPont. Hence, no abuse of discretion occurred in the phrasing of Interrogatory 3.

D. Exclusion of "Intent" Evidence

Berkley contends that the court erred in excluding evidence allegedly demonstrating that DuPont enforced its patent with intent to destroy competition.

A trial judge can and should exclude evidence when convinced that it will create a danger of prejudice outweighing its probative value.⁴⁵ The judge has wide discretion in ruling on the admissibility of evidence and his decisions thereon will not be disturbed unless there be a clear and prejudicial abuse of discretion. *Wright v. Hartford Accident & Indemnity Co.*, 580 F.2d 809, 810 (5th Cir. 1978); *Rigby v. Beech Aircraft Co.*, 548 F.2d 288, 293 (10th Cir. 1977); *Kilarjian v. Horvath*, 379 F.2d 547, 548 (2d Cir. 1967); *Great American Insurance Co. v. Horab*, 309 F.2d 262, 265 (8th Cir. 1962).

Berkley offered the deposition of Hilberg, a DuPont patent attorney, to prove that DuPont asserted its patent against Scientific Anglers, a company that had been conducting fluorescent fly line demonstrations, thus admitting that its patent encompassed the prior art fly lines of Olson and Wood and was therefore invalid.⁴⁶

⁴³ DuPont disputes Berkley's premise. However, we need not and do not decide in this case the issues of whether fraud in obtaining one claim renders the entire patent invalid or whether enforcement of a patent having claims valid and claims known to be invalid renders a patentee liable under the antitrust laws.

⁴⁴ Berkley's briefs argue error in refusal of the requested interrogatory, ignoring the effect of Instruction 21. At trial, however, Berkley recognized that juries are presumed to answer interrogatories in the light of instructions, when it successfully objected to inclusion in Interrogatory 2 of the fraud burden on the ground that the burden was already described in Instruction 21.

⁴⁵ Fed. R. Evid. 403 provides:

Rule 403. Exclusion of Relevant Evidence on Grounds of Prejudice, Confusion, or Waste of Time

Although relevant, evidence may be excluded if its probative value is substantially outweighed by the danger of unfair prejudice, confusion of the issues, or misleading the jury, or by considerations of undue delay, waste of time, or needless presentation of cumulative evidence.

⁴⁶ The examiner allowed DuPont's claims only

Hilberg's deposition was decidedly imprecise, uncertain, and seriously lacking in probative value. The two Hilberg sentences quoted by Berkley were contradicted by DuPont, which consistently maintained that it never asserted its patent against Scientific Anglers, and by Scientific Anglers itself when it told Berkley that DuPont had never asserted the patent against it.

The trial court excluded Hilberg's deposition, correctly finding it "shakey at best, its probity and relevance dubious, and potential for prejudicing DuPont's case substantial." No abuse of discretion occurred in that exclusion.⁴⁷

Berkley offered three exhibits from the deposition of Harry Haon, a DuPont employee. D-7 was a pre-suit Haon memorandum stating that Berkley was infringing, comparing the potential loss from that infringement with the cost of litigation, recommending that a legal opinion be obtained on enforceability of the patent, and, if that opinion be favorable, that suit be filed before Berkley introduced its line at a trade show and became entrenched in its infringement. E-7 was a draft of a letter to the DuPont Executive Committee, about the proposal to sue Berkley, estimating the loss from infringement, referring to a legal opinion, and viewing the cost of suit, if lost, as justified by its impact on Berkley. F-7 was a DuPont press release announcing the suit against Berkley.

[27] The exhibits contain no indication that DuPont believed its patent was invalid. Recognition that an infringement suit may not succeed simply reflects a realistic awareness of the historically low percentage of patents held valid in litigation. The potential for jury misconstruction of the Haon exhibits, which the trial court correctly described as reflecting "the internal consideration of cost versus bringing suit, which is a legitimate consideration for any patentee to engage in," would have been unacceptably prejudicial.⁴⁸

[28, 29] By definition, every good faith effort to enforce a patent involves a legitimate anticompetitive intent.⁴⁹ The Haon exhibits

after they were amended to limit them to fishing line.

⁴⁷ Berkley's briefs do not mention Scientific Anglers' denial that DuPont asserted the patent against it, nor do those briefs mention, quote, dispute, or counter the trial court's evaluation of the Hilberg deposition.

⁴⁸ The trial court correctly characterized the Haon exhibit matter as "a waste of time."

⁴⁹ If the patent be valid, the competition involves a product which by definition did not exist

indicate DuPont's consideration and recognition that a suit enforcing its patent might have adverse effects on Berkley's sales of its infringing line. There is, however, no legal obligation of patentees to withhold suit until an infringer is well along in its infringement. In all events, consideration of DuPont's anticompetitive intent is premature, absent threshold evidence that DuPont believed its patent was invalid.⁵⁰ The trial court, recognizing that distinction, correctly cited it as an additional reason for excluding the Haon exhibits:

... One reason for bifurcation was to avoid the effect of evidence relevant to one issue spilling over and coloring evidence in the other portion of the case. The Haon evidence was just such evidence.

The trial court did not abuse its discretion in excluding the Haon evidence under Fed. R. Evid. 403, on the ground that it was "prejudicial" and "not sufficiently probative."

E. Inequitable Conduct

Defining "inequitable conduct" as an intentional misrepresentation or nondisclosure to the PTO⁵¹ that, although material, did not cause issuance of the patent, and "fraud" on the PTO as a misrepresentation or nondisclosure, absent which the patent would not have issued. Berkley requested an interrogatory on inequitable conduct, the jury's affirmative answer to be used as a basis for proceeding with proof of other elements of an antitrust violation. Berkley also requested an instruction that the jury could find the DuPont patent "unenforceable" if DuPont were guilty of "inequitable conduct" before the PTO. The trial court denied both requests.

[30] Berkley's attempt to base its antitrust counterclaim on "inequitable conduct" has no basis in law.⁵² In Walker

before the inventor contributed it to the marketplace. Enforcement of the patent in that case takes nothing from the public, and nothing from the infringer to which it had any right.

⁵⁰ Citing no authority, and for the first time on appeal, Berkley argues that enforcement with anticompetitive intent alone, even if DuPont had no actual knowledge of invalidity and was not guilty of gross negligence in failing to determine invalidity, would support an antitrust claim. The argument is meritless.

⁵¹ Berkley makes no charge of specific misrepresentation, but lists seven items DuPont did not disclose to the PTO.

⁵² Berkley concedes an absence of authority, but says courts have been "equivocal" and have "indicated that logic might dictate" its position, citing only Corning Glass Works v. Anchor Hock-

Process Equipment, Inc. v. Food Machinery & Chemical Corp., 382 U.S. 172, 174, 147 USPQ 404, 405-406 (1965), the Supreme Court ruled that enforcement of a patent procured by fraud on the PTO may violate §2 of the Sherman Act if other elements necessary to a §2 case are present. Admitting that Walker Process and its progeny speak only of fraud, and acknowledging that no court has recognized "inequitable conduct" as a basis for an action under Section 2 of the Sherman Act, Berkley nevertheless argues that it is as much a violation of the Act "for a patent owner to enforce an 'unenforceable' patent against potential competitors as it is to enforce an 'invalid' patent." We disagree.

[31] A patent procured by fraud by definition would not have issued but for the misrepresentation or non-disclosure. The patent is invalid as improperly issued and the patentee has illegally received exclusionary rights he would not otherwise have. In those circumstances, as the Supreme Court held in Walker Process, the severe sanctions of the Sherman Act may be warranted.

However, where the patent was not procured through non-disclosure, the patent would properly issue and the patentee would receive no exclusionary rights to which he was not legally entitled under the patent laws. Hence, no basis exists for a charge of illegal monopolization or attempt to monopolize. Refusal to enforce the patent has been considered adequate sanction. *Mueller Brass Co. v. Reading Industries, Inc.*, 352 F.Supp. 1357, 1371, 176 USPQ 361, 371-372 (E.D. Pa. 1972), *aff'd* 487 F.2d 1395, 180 USPQ 547 (3d Cir. 1973); *SCM Corp. v. Radio Corporation of America*, 318 F.Supp. 433, 472, 167 USPQ 196, 225-226 (S.D.N.Y. 1970); *Corning Glass Works v. Anchor Hocking Glass Corp.*, *supra* note 52, at 470, 149 USPQ at 106-107.

[32] Berkley further argues, citing no authority, that if inequitable conduct standing alone is an insufficient basis for an antitrust cause of action, it is such a basis when combined with an anticompetitive intent in bringing suit on the patent. We disagree. Berkley cites excluded exhibits D-7,

Anchor Hocking Glass Corp., 253 F.Supp. 461, 470 n. 23, 149 USPQ 99, 106-107 n.23 (D.Del. 1966), *aff'd* in part, *rev'd* in part on other grounds, 374 F.2d 473, 153 USPQ 1 (3d Cir.), *cert. denied*, 389 U.S. 826, 155 USPQ 767 (1967). In that case, however, the court distinguished fraud from inequitable conduct and, referring to inequitable conduct statements, said "... there is no illegal monopoly resulting from the statements on which to base an anti-trust action."

E-7, and F-7. But those exhibits focus exclusively on events occurring *after* issuance of the patent. They bear no relation to DuPont's conduct before the PTO and, if they had been admissible, they could not bootstrap that non-fraudulent conduct, not otherwise actionable under the antitrust laws, into the more egregious Walker Process type conduct.

[33] The trial court gave no reasons for denying Berkley's requested instruction on inequitable conduct as a defense of non-enforceability.³³ Absent a ruling that the evidence was insufficient, or more prejudicial than probative, an instruction on that question would be proper.

[34] DuPont's contention that Berkley never pled an inequitable conduct defense is without merit. Berkley's inequitable conduct theory differs from its expressly pled allegation of fraud only in the degree of materiality of the information allegedly withheld from the PTO. The pleadings placed DuPont on notice of the type of conduct that would be litigated, and that is all that is required. See *Conley v. Gibson*, 355 U.S. 41, 47 (1957).

[35] This circuit has recognized that inequitable conduct short of fraud can be a defense in a patent infringement suit. *Pfizer, Inc. v. International Rectifier Corp.*, 538 F.2d 180, 185, 190 USPQ 273, 277 (8th Cir. 1976), *cert. denied*, 429 U.S. 1040, 192 USPQ 543 (1977). To make out a case of inequitable conduct Berkley must prove, by "clear, unequivocal and convincing evidence," *Pfizer, Inc. v. International Rectifier Corp.*, *id.* at 187, 190 USPQ at 279, that DuPont's conduct made it impossible for the PTO to fairly assess the patent application against the prevailing statutory criteria. In *re* Multidistrict Litigation Involving Frost Patent, 540 F.2d 601, 604 n.9, 191 USPQ 241, 243 n.9 (3d Cir. 1976), and that it involved "some element of wrongfulness, wilfulness or bad faith." *Pfizer, Inc. v. International Rectifier Corp.*, *supra* at 186, 190 USPQ at 278. If the information be irrelevant, its innocent or negligent misrepresentation or non-disclosure, whether or not intentional, does

³³ In denying Berkley's motion for a new trial (i.e., a trial of its antitrust claim) on inequitable conduct, the trial court stated:

... Although inequitable conduct is a viable theory in patent law, it had no place in this case as it was never pleaded and even if pleaded would only have gone to the enforcement of the patent and not to the antitrust issues. The jury held the patent unenforceable.

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not amount to inequitable conduct. *Pfizer, Inc. v. International Rectifier Corp.*, supra, at 186, 190 USPQ at 278; *Corning Glass Works v. Anchor Hocking Glass Corp.*, supra note 52, at 471 n.27, 149 USPQ at 107 n.27.

[36] A strong caveat was raised by this court in *Pfizer, Inc. v. International Rectifier Corp.*, supra at 196, 190 USPQ at 286-287:

An infringement defendant in complex litigation should not be permitted to sidestep these main issues by nit-picking the patent file in every minute respect with the effect of trying the patentee personally, rather than the patent. A patentee's oversights are easily magnified out of proportion by one accused of infringement seeking to escape the reach of the patent by hostilely combing the inventor's files in liberal pretrial discovery proceedings. Unjustified damage to professional and social reputations can result, as here, without fostering any corresponding public benefit in the form of inhibiting future improvident grants of patent monopolies.

[37] Thus Berkley may face a heavy burden in establishing inequitable conduct on the evidence concerning non-disclosures to the PTO. Nonetheless, absent the trial court's determination that the evidence was legally inadequate, or more prejudicial than probative, Berkley had a right to jury determination of whether the information not disclosed was sufficiently relevant to meet the inequitable conduct standard of materiality but not sufficiently relevant to meet the fraud standard of materiality.⁵⁴ No useful purpose would be served by a review of that evidence here.⁵⁵ If the inequitable conduct

⁵⁴ The jury asked the court whether a finding of fraud would "open the door for a fraud case" against DuPont. The court correctly answered that the jury should ignore consequences of its findings.

⁵⁵ Except, perhaps, to mention that evidence submitted to show non-utility has no place at retrial.

DuPont argues the evidence was not probative of inequitable conduct, because the information not disclosed was irrelevant or less relevant than that which was disclosed, and points to an absence of evidence of bad faith. These are matters, however, for the trial court in the first instance, or, if the evidence be admitted, for a jury following proper instructions.

DuPont does not specifically argue absence of error in refusing an instruction on enforceability. It says the only question to which inequitable conduct may relate is Berkley's demand for attorneys' fees, denied by the trial court because

defense be renewed at retrial, the trial court, with the above guidance, will determine in its discretion the admissibility of evidence presented. It is sufficient on this appeal to hold that the trial court erred in refusing, without explanation, to instruct the jury on the defense of inequitable conduct. Berkley may renew this defense against enforcement at retrial.

F. Dismissal of the Antitrust Counterclaim

Berkley says it didn't get its day in court on its counterclaim. We disagree.

Berkley sought to prove two species of antitrust violation, one based on fraudulent conduct before the PTO, the other based on bringing suit with knowledge that the patent was invalid. Proof of one of those threshold allegations was essential to Berkley's antitrust cause of action.

The court gave Berkley a full and fair trial on both threshold questions and submitted them specially to the jury in Interrogatories 2 and 3.⁵⁶ When the jury answered "no" to both, establishing that DuPont did not obtain the patent by fraud and did not enforce the patent knowing it to be invalid, Berkley's antitrust counterclaim was necessarily stripped of all foundation and support.

As indicated supra, Berkley's allegations of gross negligence were unsupported by the evidence. Its allegations of inequitable conduct and anticompetitive intent will not support its antitrust counterclaim.

Berkley having had its day in court, the trial court properly dismissed its antitrust counterclaim.

Summary

The jury having been presented with substantial evidence on utility, that issue having been one of the predominant matters litigated, it is probable if not certain that the jury based its verdict for Berkley on a belief that DuPont's invention lacked utility. Consequently, the error of submitting the utility issue to the jury was so highly prejudicial to DuPont as to warrant a new trial on the issue of patent validity:

The error in submitting to the jury the lack-of-novelty defense based on the McCoy

DuPont's acts in pursuing and asserting its patent were not so "reckless or fraudulent" as to make this an exceptional case. But that is a different question from whether Berkley had a right to have its nondisclosure evidence go to the jury.

⁵⁶ Berkley's brief is thus mistaken in stating "the jury heard the patent infringement case but never reached the antitrust issues." The jury reached and decided the bedrock antitrust issues.

line, that issue having also received extensive treatment at trial, would, standing alone, warrant a new trial on patent validity.

The combination of the utility and McCoy line prejudicial errors renders the need for a new trial compelling on patent validity.⁵⁷

The lack-of-novelty defense based on the Cohantic line, and the obviousness defense, present proper jury questions. On retrial, the court need litigate only those issues respecting the validity of the DuPont patent.

The trial court committed no reversible error in (1) refusing an instruction and interrogatory on gross negligence; (2) instructing on fraud; (3) phrasing Interrogatories 2 and 3; (4) excluding the Hilberg deposition and Haon exhibits; (5) refusing an instruction on inequitable conduct as a basis for an antitrust violation; and (6) dismissing the antitrust counterclaim.

The jury's findings that DuPont did not obtain the patent by fraud and did not enforce the patent knowing it to be invalid were supported by the evidence.

The trial court erred in refusing to instruct the jury on inequitable conduct as a defense of unenforceability to DuPont's patent infringement suit.

Conclusion

The dismissal of Berkley's antitrust counterclaim is affirmed.

The judgment holding the DuPont patent invalid is vacated, and the case is remanded for a new trial on the issues of validity and enforceability of the DuPont patent. Three defenses appear appropriate, namely, that (1) the invention was anticipated by the Cohantic Line; (2) the invention would have been obvious to one skilled in the art at the time it was made; and (3) the patent was rendered unenforceable by DuPont's inequitable conduct before the PTO.⁵⁸

⁵⁷ We need not consider whether the French line and presumption of validity errors were so prejudicial as to independently warrant a new trial on patent validity.

⁵⁸ Most references to retrial before a jury herein are applicable to retrial before a judge. Though piecemeal litigation is not the norm, consideration might be given to sequential trials under Rule 42(b), Fed. R. Civ. P. If defense (1) is sustained, trial is unnecessary on (2) and (3). If defense (1) fails, but (2) is sustained, (3) is unnecessary. Similarly, a successful defense (3) would render trial of (1) and (2) unnecessary, the validity of a patent expired and unenforceable being moot. The sequence and grouping of issues tried first might turn on the estimated time to try

District Court, N. D. Georgia, Atlanta Div.

Robert B. Vance & Associates,
Inc. et al. v. The Baronet Corporation et al.

No. C76-1152A
Decided Dec. 21, 1979

TRADEMARKS

1. Defenses — Trademark cases (§30.20)

Registration — Effect (§67.747)

Registration — Incontestability (§67.751)

Mark's registration serves as conclusive evidence of registrant's exclusive right to use it, as general rule, if it has become incontestable under 15 U.S.C. 1065; Lanham Act sets forth seven defenses that may be raised in infringement action in which registrant's mark has become incontestable; 15 U.S.C. 1115(b)(4) provides defense to infringement action if use of name, term, or device charged to be infringement is use, otherwise than as trade or service mark, of term or device that is descriptive of and used fairly and in good faith only to describe to users, goods or services of such party.

2. Marks and names subject to ownership — Descriptive — In general (§67.5071)

Marks and names subject to ownership — Descriptive — How determined (§67.5073)

Marks and names subject to ownership — Suggestive (§67.528)

Four categories of trademarks are generic, descriptive, suggestive, and arbitrary or fanciful; generic mark refers to common descriptive name of article or substance; descriptive mark conveys clear idea of characteristics or qualities of goods; suggestive mark falls somewhere in between descriptive marks and those that can be termed fanciful or arbitrary; suggestive term merely suggests characteristics of goods, rather than describing them, and effort of

each. Separate appeals are conceivable, but the clear-cut clarity of each might result in a net gain in judicial economy.

The jury did not answer the interrogatories submitted to it on DuPont's damages and Berkley's intentional infringement. Though vigorously argued here, these issues are not ripe for resolution on this appeal. They may be presented at retrial.

Court of Appeals, Federal Circuit**Envirotech Corporation
v. Al George, Incorporated et al**

No. 83-1107

Decided Mar. 19, 1984

PATENTS**1. Infringement — Law or fact question
(§39.60)****Infringement — Tests of — Comparison
with claim (§39.803)**

Finding of infringement depends on whether accused device falls within scope of asserted claims as properly interpreted; patented invention as indicated by language of claims must first be defined — question of law — and then trier must judge whether claims cover accused device — question of fact.

2. Pleading and practice in courts — Burden of proof — Infringement (§53.134)

Patent owner must show by preponderance of evidence that accused has infringed his patent.

3. Court of Appeals for the Federal Circuit — Weight given decision reviewed (§26.57)**Pleading and practice in courts — Motions — In general (§53.631)**

Judge on JNOV motion in patent infringement suit — and CAFC on review — must ascertain whether there was substantial evidence of such quality and weight that reasonable and fair-minded men in exercise of impartial judgment could reasonably return verdict for non-moving party.

4. Construction of specification and claims — Claim defines invention (§22.30)**Infringement — Tests of — Comparison
with claim (§39.803)**

Resort must be had in first instance to words of claim which define metes and bounds of invention; if accused matter falls clearly within terms of claim, infringement is normally made out; said another way, what is patented must first be defined.

5. Construction of specification and claims — Defining (§22.45)

Words in claim will be given their ordinary and accustomed meaning unless it appears that inventor used them differently.

6. Pleading and practice in courts — Jury trial — Validity and infringement (§53.577)

It was up to jury to determine whether portion of accused device is in fact functional.

7. Infringement — Law or fact question (§39.60)

Whether defendants infringed claims of patent is factual issue.

8. Construction of specification and claims — By specification and drawings — In general (§22.251)

Terms of claims are best construed in light of specification and circumstances that surround patent at its inception.

9. Courts of Appeals — Weight given findings of District Court — Validity and infringement (§29.359)**Infringement — Law or fact question (§39.60)****Infringement — Substitution of equivalents (§39.751)**

Accused device may infringe if it performs substantially same function in substantially same way to obtain same result as claimed apparatus; finding of equivalence is determination of fact that may be based on proof by testimony of experts, and should be disturbed only as allowed by general principles governing appellate review.

10. Jurisdiction of courts — Declaratory judgment — Actual controversy (§43.303)

Although CAFC affirmance of judgment of non-infringement may render academic, validity of patents at issue, if defendants on remand seek to continue with their claim of invalidity, court below and parties will have to face problems whether constitutional "case or controversy" still exists between present parties, and if it does, whether it is appropriate to issue declaratory judgment.

11. Pleading and practice in courts — Jury trial — In general (§53.571)**Presumption from patent grant — Patent Office consideration of prior art (§55.5)**

Instruction that "as to information or documents (prior art) which you find from file wrapper to not have been considered by the examiner and which you find to teach an invention closer to or more like that claimed *** no such presumption (of validity) ex-

ists," is improper; presumption does not change upon introduction of that art, or at any other time; it is upon introduction of more relevant art that challenger's burden of persuasion may be more easily carried.

12. Patentability — Utility (§51.75)

Fact that invention has only limited utility and is only operable in certain applications is not grounds for finding lack of utility; some degree of utility is sufficient for patentability; further, defense of non-utility cannot be sustained without proof of total incapacity.

13. Pleading and practice in courts — Jury trial — In general (§53.571)

Submission of obviousness question to jury should preferably be accompanied by specified "interrogatories" designed to elicit responses to all factual inquiries enumerated in *Graham v. John Deere Co.*, 148 USPQ 459; proper instructions based on those inquiries should always be given; "interrogatory" is inapt instruction that reads, "Do you find that defendants have proved by clear and convincing evidence that the differences between the patent claims and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains;" jury's answer — "Was obvious" — is type of special verdict on issue of obviousness.

14. Court of Appeals for the Federal Circuit — Pleading and practice (§26.57)

CAFC must be satisfied that party challenging validity of patent has properly carried its burden of overcoming presumption of validity; absent such satisfaction, CAFC merely vacates and remands on issue of validity, since court never "declares" patent valid; CAFC leaves initially to district court extent to which new trial is required if issue of patent invalidity is pursued on remand.

Particular patents — Flotation Processes and Devices

4,110,210, Degner and Colbert, Dispersed Gas Flotation Process, holding of noninfringement of claims 1, 14, and 15, affirmed; holding of invalidity vacated.

4,226,706, Degner and Colbert, Dispersed Air Flotation Machine, holding of noninfringement of claims 1, 10, 11 and 12, affirmed; holding of invalidity vacated.

Appeal from District Court for the Western District of Louisiana.

Action by Envirotech Corporation, against Al George, Incorporated, and Monosep, Inc., for patent infringement, in which defendants counterclaim for declaration of patent invalidity and noninfringement. From judgment for defendants, plaintiff appeals. Affirmed in part, vacated in part, and remanded; Baldwin, Circuit Judge, specially concurring, with opinion.

V. Bryan Medlock, Jr., Dallas, Tex., for appellant.

H. Coke Wilson, Houston, Tex. (Thomas Marsteller, Houston, Tex., of counsel) for appellee.

Before Davis, Baldwin and Kashiwa, Circuit Judges.

Davis, Circuit Judge.

Envirotech Corporation (Envirotech) appeals from a judgment, after a jury trial, of the United States District Court for the Western District of Louisiana holding that claims 1, 14 and 15 of its U.S. Patent 4,110,210 ('210) ("Dispersed Gas Flotation Process") and claims 1, 10, 11 and 12 of its U.S. Patent 4,226,706 ('706) ("Dispersed Air Flotation Machine") are invalid and not infringed by appellees Al George, Incorporated and Monosep, Inc. (Monosep). We affirm in part, and vacate and remand in part.

I

Background

A. The General Technology

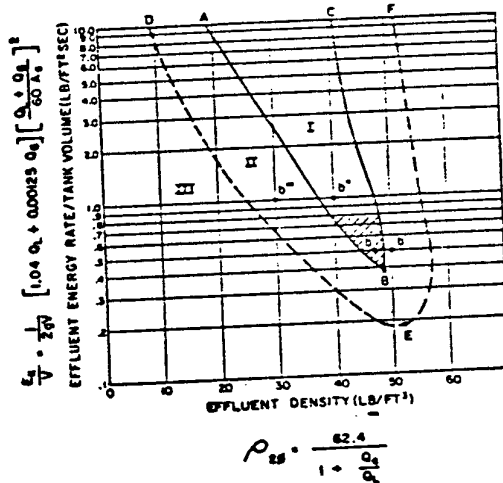
The Envirotech patents involve separation of materials having different densities by ejecting minute gas bubbles into a tank containing a two-component fluid or solid/fluid "slurry." The bubbles attach to the particles to be separated and provide sufficient buoyancy so that both particle and bubble float to the tank surface to form a froth that is skimmed from the surface — thus, the term "flotation separation." Typical flotation separation devices use a nozzle extending beneath the tank's surface in order to introduce bubbles into the tank by ejecting a mixture of air and water as a two-phase (gas and liquid) effluent.

In order to have successful flotation separation, two principal conditions must exist. First, the surface of the liquid in the tank must be relatively smooth because turbulence will dislodge the particles from their bubbles

causing them to sink back into the fluid. Second, the bubbles must be dispersed throughout the tank in order to come into contact with all of the particles to be separated. In the past, dispersion was achieved by using various types of impellers placed at the bottom of the tank, using multiple nozzles in each tank, or using baffles.

B. The Method of the '210 Patent

The '210 patent discloses a flotation separation method in which a two-phase effluent is ejected from a single nozzle into a tank in such a fashion that the desired conditions of good bubble distribution and a smooth non-turbulent surface may be obtained throughout a range of tank sizes without the use of baffles, impellers or multiple nozzles. The invention recognized that one could obtain these optimal conditions with a certain effluent density (gas-liquid ratio) and a certain energy rate per tank volume (velocity of the effluent). Figure 2 of the '210 patent graphically illustrates this energy/density relationship for a wide range of tank sizes:



The curves set forth in Fig. 2, supra, divide the graph into Regions I, II and III. If a nozzle and tank combination are designed to operate within Region I then the two conditions for optimal separation can be obtained. Operation within Region III lacks these two conditions, and Region II is a gray area. Claim 1 of the '210 patent is representative:

1. A dispersed gas flotation process wherein hydraulic effects are used to disperse gas bubbles throughout a contained liquid body with a free surface, said process comprising pumping a two-phase fluid into the liquid

body through an ejection device with the density and the kinetic energy rate of the ejected fluid per unit volume of the contained body at the point of ejection being defined by a point on the graph of FIG. 2 within the area encompassed by Region 1.

According to the teachings of the patent, to calculate effluent density the designer need know only the flow rate of gas through the nozzle (Q_g) and the flow rate of the liquid through the nozzle (Q_l). To calculate the effluent energy rate per tank volume, the designer must know the tank volume (V), the flow rate of the gas through the nozzle (Q_g), the flow rate of the liquid through the nozzle (Q_l), and the area of the effluent being ejected into the tank at the "point of ejection" (A_e).

C. The Apparatus of the '706 Patent

The '706 patent discloses a flotation separator having a series of tanks arranged in line so that the contaminated fluid can be treated in stages from one tank to another. As shown in Figure 2 of the '706 patent, reproduced below, each flotation tank 13 has one nozzle 20 (extending below the surface of the liquid) which ejects the two-phase effluent. The froth, which is subsequently formed by the bubble-carrying particles, is then skimmed off by paddle wheels 14, 14a.

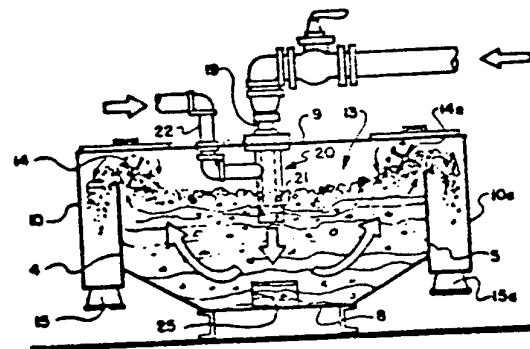
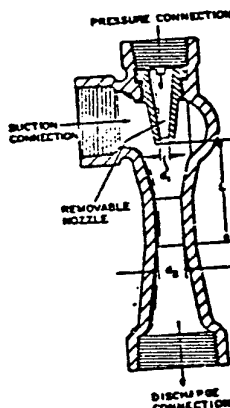


FIG. 2

The claims of the '706 patent require that each nozzle have a "hollow tubular expansion chamber member" through which the two-phase effluent is discharged. The claimed pipe-within-a-pipe configuration comprises a smaller inner pipe which carries the liquid into a larger outer pipe creating a vacuum which sucks air down the outer pipe into the expansion chamber where it is mixed with the liquid from the inner pipe. The '706 patent expressly incorporates in the specification the types of "converging-diverging" nozzles included in the '210 patent. (Column 4, lines 31-33.)

D. The Defendants' Alleged Infringing Device

The defendants' accused device, marketed under the name "Multisep", has a series of tanks, each having a single off-the-shelf nozzle (also called an "eductor") where liquid is pumped and air is drawn in. The resulting two-phase effluent (air and liquid) is then ejected into the tank filling it with small bubbles. Defendants' eductor nozzle, illustrated below, normally used to pump two liquids, is of the converging-diverging type with a cylindrical throat portion and a flared end portion.



E. The Parties' Contentions Below

The defendants stipulated that if their device operated within Region I of the '210 patent graph, they would be infringing that patent. As already mentioned, to ascertain the effluent energy rate, the area (A_e) at the "point of ejection" from the nozzle must be known. However, the parties disagree about the measurement of the exit diameter of the effluent stream from the defendants' nozzle — a critical difference which affects infringement. Because the defendants' nozzle has a flared end, the diameter in the throat is smaller than the diameter at the end of the nozzle. Defendants say that, if the effluent fills up the flared end due to back pressure, then the larger end diameter should be used, and thus their process would fall completely off the '210 patent graph. The flared end is said to be considered "functional" if the effluent fills up the flare, meeting the tank fluid at the end of the nozzle. Under this construction, the "point of ejection" is at the nozzle's end. On the other hand, if the flared end is not functional, as plaintiff says, the throat diameter is to be used because the effluent exits in a column which does not contact the walls of the flared end, and, thus, defendants' machine

falls within Region I of the '210 patent graph. Under this latter construction, plaintiff contends that the flared end is merely non-functional and cosmetic, and the "point of ejection" is at the throat.

Defendants also stipulated that their machines included many of the elements of the '706 patent. They contend, however, that neither plaintiff's claimed "hollow tubular expansion chamber member" nor the incorporated '210 nozzles read on their converging-diverging off-the-shelf eductor nozzle.

F. Proceedings Below

Plaintiff appellant Envirotech sued defendant appellees Monosep for infringement of claims 1, 14 and 15 of the '210 patent and claims 1, 10, 11 and 12 of the '706 patent. Defendants Monosep counterclaimed for a declaratory judgment of invalidity and non-infringement. The action was tried before a judge and a jury from March 7, 1983 through March 11, 1983. The judge gave very general instructions on the law of patents and evidence, and then gave very general special interrogatories, simply asking whether each invention was or was not "novel", "new", "useful", "obvious", whether each patent "distinctly claims the inventions", whether each invention "has been adequately described," and also whether defendants infringed the patents. In answer to these summary interrogatories the jury found the claims at issue in the '210 patent "novel", "new", "not useful", "obvious", "fails" to "particularly point out and distinctly claim the subject matter", "fails" to adequately describe the subject matter," and not infringed. Further, the jury found the claims at issue in the '706 patent "novel", "obvious", and not infringed. Without further comment, the judge adopted the jury's findings and adjudged the claims at issue in the '210 patent invalid under 35 U.S.C. §§101, 103, 112, and not infringed, and the claims at issue in the '706 patent

¹ We are unsure what "new" was intended to mean, as distinguished from "novel".

² On the last day of trial, defendants were granted their motion to amend the pleadings to add the defense of lack of utility. The defense seems to be one of inoperability of the '210 patent graph for failing to set forth critical limitations as a result of a lack of exact correlation between the patentee's experimental data and the actual graph in the patent.

^{3,4} These latter two defenses — enablement and definiteness — are based on alleged ambiguity of the claimed "point of ejection".

invalid under 35 U.S.C. §103 and not infringed. The proposed findings of fact and conclusions of law of both parties were rejected.

The plaintiff subsequently made a motion for judgment notwithstanding the verdict and, in the alternative, for new trial based on the lack of substantial evidence to support the jury's verdict. By order, but without any memorandum, the district court denied the motion.

On this appeal, both the issues of validity and infringement are controverted. We consider them in the reverse order.

II

Infringement

[1,2] In general, a finding of infringement depends on whether the accused device falls within the scope of the asserted claims as properly interpreted. *Kalman v. Kimberly-Clark Corp.*, 713 F.2d 760, 770, 218 USPQ 781, 788 (Fed. Cir. 1983). The patented invention as indicated by the language of the claims must first be defined (a question of law), and then the trier must judge whether the claims cover the accused device (a question of fact). See *Fromson v. Advance Offset Plate, Inc.*, 720 F.2d 1565, 1569, 219 USPQ 1137, 1140 (Fed. Cir. 1983); *SSIH Equipment S.A. v. USITC*, 718 F.2d 365, 375, 218 USPQ 678, 688 (Fed. Cir. 1983). The patent owner must show by a preponderance of the evidence that the accused has infringed his patent. *Hughes Aircraft v. United States*, 717 F.2d 1351, 1361, 219 USPQ 473, 480 (Fed. Cir. 1983); *SSIH*, supra; *Chisum*, Patents, 18.06[1] (1983).

[3] This was a jury case, and the judgment below must be scrutinized by the rules applicable to such jury cases. As the appellate court, we review the jury's findings of fact, (i.e., here that plaintiff failed to meet this burden) in light of the district court judge's denial of plaintiff's JNOV motion. A court cannot merely substitute its view for that of the jury's when reviewing questions of fact. Instead, the guidelines for considering motions for judgment notwithstanding the verdict are: (1) all of the evidence must be considered; (2) in a light most favorable to the non-moving party; (3) drawing all reasonable inferences favorable to that party; (4) without making determinations of credibility of the witnesses. *Connell v. Sears, Roebuck & Co.*, 722 F.2d 1542, 1546, 220 USPQ 193, 197 (Fed. Cir. 1983); *Railroad Dynamics, Inc. v. A. Stucki Company*, Appeal Nos. 83-951,

961, slip op. at 11-12, 220 USPQ at 936 (Fed. Cir. January 25, 1984). The judge on a JNOV motion (and this court on review) must ascertain whether there was "substantial evidence of such quality and weight that reasonable and fair-minded men in the exercise of impartial judgment could reasonably return a verdict for the non-moving party". *Wyatt v. Interstate & Ocean Transport Co.*, 623 F.2d 868, 891 (4th Cir. 1980). As this court pointed out in *Connell and Railroad Dynamics*, supra, these guidelines are applicable to patent infringement suits. Thus, the precise issue before us on infringement is whether there was substantial evidence to support the jury's factual finding that Envirotech failed to prove infringement by a preponderance of the evidence.

The '210 Method Patent

[4,5] It is elementary that resort must be had in the first instance to the words of the claim which define the metes and bounds of the invention. If the accused matter falls clearly within the terms of the claim, infringement is normally made out. *Graver Tank & Mfg. Co. v. Linde Air Products Co.*, 339 U.S. 605, 607, 85 USPQ 328, 330 (1950); *Smith International, Inc. v. Hughes Tool Company*, 718 F.2d 1573, 1579, fn. 2, 219 USPQ 686, 691 fn. 2 (Fed. Cir. 1983). Said another way, what is patented must first be defined. Words in a claim "will be given their ordinary and accustomed meaning, unless it appears that the inventor used them differently". *Universal Oil Products Co. v. Globe Oil & Refining Co.*, 137 F.2d 3, 6, 54 USPQ 504 (7th Cir. 1943), aff'd, 322 U.S. 471, 61 USPQ 382 (1944).

Representative claim 1 of the '210 patent describes, inter alia, "[a] dispersed gas flotation process wherein *** the density and the kinetic energy rate *** per unit volume *** at the point of ejection ***" (Emphasis added.) The area ("per unit volume") of the effluent discharged from the nozzle is measured at the "point of ejection". The diameter of defendant's nozzle used to measure that area depends upon the question of where the "point of ejection" exists in the diverging section of the nozzle; thus, infringement here depends on this "point of ejection".

Plaintiff appellant argues that the "point of ejection" describes the point where the effluent meets the tank fluid. Defendant appellees argue that ejection occurs at the end of the nozzle. In effect both are saying the same thing. The claim language may be understood, as appellees contend, to define the "point of ejection" as the point where the

effluent effectively leaves the piece of hardware. However, this does not rule out an interpretation that the "point of ejection" may be at the throat area as Envirotech contends. The flared end of defendant's nozzle may not be functional, and thus the effluent effectively leaves the nozzle at the throat area. We therefore construe the claim to describe the "point of ejection" as the point where the effluent effectively meets the tank liquid.

[6] It was up to the jury to determine whether the defendant's flared end is in fact functional or whether the effluent effectively meets the tank fluid in the throat area of the nozzle. As applied to the evidence, the significance of the latter determination if made, as we have pointed out, would be that defendants' process would fall within Region I of the '210 graph.

Envirotech asserts that the flared end is functional only when there is enough back pressure in the flare to fill up the flared end. To support its contention, Envirotech called as its expert witness, Dr. Bourgoyne, professor and chairman of the petroleum engineering department at Louisiana State University. During his testimony, Dr. Bourgoyne offered a demonstration of a plastic replica of defendants' device in an attempt to show that the effluent ejected in a column never touched the sides of the flared end.⁵ Bourgoyne stated that the plastic replica would behave the same as defendants' cast-iron eductor. The entire jury stepped up to observe both the eductor and the fluid flow seen through the plastic.

Defendant called Dr. Muster, professor of mechanical engineering at the University of Houston. He asserted that one cannot tell precisely where the "point of ejection" would be because of changing conditions in a turbulent zone. If anything, he argued, the most sensible "point of ejection" would be where the effluent exited at the end of the nozzle. In addition, defendants read into the record the deposition of Dr. Colbert, one of the patentees, which appears to say that, with a nozzle of defendants' type, the "point of ejection" is at the end of the nozzle.

[7] The jury, faced with a clear conflict in evidence, returned a finding that defendants had not infringed the claims of the '210 patent. This is plainly a factual issue. Based on the testimony of the two experts and the in-court demonstration we have to agree that

there was substantial evidence on which the jury could make a finding of non-infringement. Accordingly, we hold that plaintiff failed to prove by a preponderance of the evidence infringement of claims 1, 14 and 15 of the '210 patent.

The '706 Apparatus Patent

Once again we must start with the language of the claims. The element in the claims which is dispositive requires, inter alia, a:

"fluid ejection device including: (i) a hollow tubular expansion chamber member which has an open end through which the mixed fluid is ejected into a liquid * * *" (emphasis added)

[8] Thus, the precise issue for literal infringement of the '706 patent is whether defendants' converging-diverging eductor comprises a "hollow tubular expansion chamber member" within the meaning of the claims. The terms of claims are best construed in light of the specification and the circumstances which surround the patent at its inception. *Fromson v. Advance Offset Plate, Inc.*, 720 F.2d 1565, 1569, 219 USPQ 1137, 1140 (Fed. Cir. 1983); *Autogiro Co. v. United States*, 384 F.2d 391, 397, 155 USPQ 697, 702 (Ct. Cl. 1967). Adverting to the specification, appellant argues that the fluid ejection devices (nozzles) claimed in the '210 patent are incorporated by reference into the '706 patent specification, and accordingly the claims read on defendants' devices. Claim 12 of the '210 patent recites a converging-diverging type of nozzle to be used with the process of claim 1 in the '210 patent. Claim 14 of the '210 patent recites an expansion chamber type of device also to be used with the process of claim 1. Those recitations of the '210 patent are part of the '706 patent specification. See *General Electric Co. v. United States*, 572 F.2d 745, 758, 198 USPQ 65, 76 (Ct. Cl. 1978); *In re Schaumann*, 572 F.2d 312, 317 n. 11, 197 USPQ 5, 9 fn. 11 (CCPA 1978); *Velo-Bind, Inc. v. Minn. Mining & Mfg. Co.*, 647 F.2d 965, 968, 211 USPQ 926, 929 (9th Cir. 1981). However, in connection with infringement of the '706 patent, two essential points must be remembered: first, it is still the claims of the '706 patent which must be found infringed; and second, incorporation by reference can only aid in the construction of the '706 claims.

On the basis of the incorporation by reference, appellant argues that its pipe-within-a-pipe configuration which requires a "hollow tubular expansion chamber member" includes the converging-diverging type of noz-

⁵ Defendants attacked the value of the demonstration on the lack of "threads" at the end of the nozzle. Dr. Bourgoyne subsequently held the same demonstration with threads cut into the flared end — akin to defendants' nozzle.

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III

Validity

zle.⁶ But defendants' expert, Dr. Muster, testified that the Monosep eductor does not have a hollow straight tubular member. Even the throat portion of the eductor, which is straight over a small length relative to the entire nozzle, would not qualify. Not only was the jury aware of Dr. Muster's testimony but there was the in-court demonstration of a purported replica. Although the purpose of the demonstration was to show where the "point of ejection" was within the meaning of the '210 patent, the jury could use the demonstration to ascertain whether there was an expansion chamber within the meaning of the '706 patent. Thus, there was substantial evidence of such quality and weight to support a non-infringement verdict in favor of Monosep. Plaintiff did not meet its burden to prove literal infringement of the '706 patent.

[9] Alternatively, plaintiff alleges infringement under the doctrine of equivalents. This doctrine is usually asserted when actual literal infringement is not present. See *Hughes Aircraft Company v. United States*, 717 F.2d 1351, 1361, 219 USPQ 473, 480 (Fed. Cir. 1983). An accused device may infringe "if it performs substantially the same function in substantially the same way to obtain the same result" as the claimed apparatus. *Graver Tank & Mfg. Co. v. Linde Air Products Co.*, 339 U.S. 605, 608, 85 USPQ 328, 330 (1950). A finding of equivalence is a determination of fact which may be based on proof by testimony or experts, and should only be disturbed as allowed by the general principles governing appellate review. *Id.* at 609-610, 85 USPQ at 630, 631.

Even if we assume that the '210 claimed nozzles were incorporated by reference into the '706 patent, plaintiff has still failed in its burden to prove infringement by equivalence. The jury had before it both patents and was aware of the incorporation by reference. In addition, the judge gave the correct definition of the doctrine of equivalents to the jury. Testimony by Dr. Muster showed, contrary to appellant's assertions, that it was disputable whether a pipe-within-a-pipe nozzle and converging-diverging nozzle perform the same function in the same way to obtain the same result. Accordingly, we cannot say that there was not substantial evidence on which the jury could base its verdict of non-infringement. The result is that Envirotech failed to prove by a preponderance of the evidence Monosep's infringement of claims 1, 10, 11 and 12 of the '706 patent.

⁶ The expansion chamber is where the gas mixes with the liquid before being discharged as a two-phase effluent.

[10] Our affirmance of the judgment of non-infringement may render academic (for this case) the validity of the '210 and '706 patents. But defendant-appellees have sought a declaratory judgment of invalidity, and may wish to pursue it on the remand which we consider necessary on the issue of validity.⁷

We consider that serious errors vitiated the district court's determination of invalidity. The judge gave erroneous jury instructions which marred the jury's consideration of the factual and legal issues bearing on validity.

[11] For one thing, the judge instructed:

"as to information or documents (prior art) which you find from the file wrapper to not have been considered by the examiner and which you find to teach an invention closer to or more like that claimed *** such presumption (of validity) exists ***"

This instruction is not proper. The "presumption does not change upon introduction of that art, or at any other time." *Connell v. Sears, Roebuck & Co.*, 722 F.2d 1542, 1549, 220 USPQ 193, 200 (Fed. Cir. 1983). See also, *American Hoist & Derrick Co. v. Sowa & Sons, Inc.*, Appeal Nos. 83-555, 83-564, slip op. at 10-16, 220 USPQ 763 (Fed. Cir., January 12, 1984). It is upon the introduction of more relevant art that the challenger's burden of persuasion may be more easily carried.⁸

[12] The district court also erred in its handling of the utility defense. Defendants

⁷ If defendants do seek to continue with their claim of invalidity, the court below and the parties will have to face the problems whether a constitutional "case or controversy" still exists between the present parties, and if it does whether it is appropriate to issue a declaratory judgment in this case. We intimate no opinion on either of these questions.

⁸ The most relevant prior art defendants offered were U.S. Patent No. 2,938,629 to Hollingsworth entitled "Concentration of Commuted Materials", and U.S. Patent No. 3,446,353 to Davis entitled "Method and Apparatus for Froth Flotation". Although these patents were primarily offered at trial to show invalidity of the '706 patent, their relationship to the '210 patent was also discussed. The Hollingsworth patent which also addresses flotation devices, suggests the advantage of multiple nozzles in each tank. (Column 3, lines 25-29.) At trial, and then in their closing argument, defendants pointed toward language which they contend suggest single nozzle operation. (Column 6, lines 65-71 and Column 9, lines 66-71, respectively.) The Davis patent points out the effect power (kinetic energy rate) and tank volume have on bubble distribution and the tank surface.

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argued that the alleged critical limitations represented by Region I of the '210 patent graph do not truly delineate the desired operating conditions described in the patent. In addition, they alleged a lack of exact correlation between experimental graphs and the actual patent graph. As we understand them, these defenses are based on the specification's alleged failure to disclose adequately to one ordinarily skilled in the art "how to use" the invention without undue experimentation — usually considered the "how-to-use" defense under 35 U.S.C. §112. See, *Chisum, Patents*, 7.03[6] (1983). However, such assertions have been applied to an argument for lack of utility under 35 U.S.C. §101 when there is a complete absence of data supporting the statements which set forth the desired results of the claimed invention. See, e.g., *In re Ruskin*, 354 F.2d 395, 148 USPQ 221 (CCPA 1966) (specification devoid of any quantitative data). The trial judge did nothing to dispel any confusion between §101 and §112. He even added to the problem by first allowing defendants' last amendment of the pleadings to include a lack of utility defense,⁹ and then merely quoting from §101 in his charge. Even if defendants' argument of inadequate experimentation or inexact correlation were to apply, the fact that an invention has only limited utility and is only operable in certain applications is not grounds for finding lack of utility. *Raytheon Co. v. Roper Corp.*, 724 F.2d 951, 958-59, 220 USPQ 592, 598 (Fed. Cir. 1983); *Carpet Seaming Tape Licensing Corp. v. Best Seam, Inc.*, 694 F.2d 570, 578, 216 USPQ 873, 880 (9th Cir. 1982). Some degree of utility is sufficient for patentability. *E. I. duPont de Nemours & Co. v. Berkley and Co.*, 620 F.2d 1247, 1260, fn.17, 205 USPQ 1, 10 (8th Cir. 1980). Further, the defense of non-utility cannot be sustained without proof of total incapacity. *Id.*

Perhaps the most glaring defect arises from the defective charge on the issue of obviousness. In his instructions to the jury the judge did not set forth the trilogy of factual inquiries outlined in *Graham v. John Deere Co.*, 38 U.S. 1, 148 USPQ 459 (1966): (1) the scope and content of the prior art; (2) the differences between the prior art and the claims at issue; and (3) the level of ordinary skill in the art. Nor was the jury called upon to answer special interrogatories on these inquiries, or to give special verdicts thereupon.

[13] Submission of the obviousness question to the jury should preferably be accom-

panied by specified interrogatories designed to elicit responses to all the factual inquiries enumerated in *Graham*. Proper instructions based on those inquiries should always be given.¹⁰ See *Connell*, supra, 722 F.2d at 1547, 220 USPQ at 197; *American Hoist & Derrick Co. v. Sowa & Sons, Inc.*, supra, slip op. at pp. 18-19, 220 USPQ at 771; *Railroad Dynamics, Inc. v. A. Stucki Company*, supra, slip op. at 17 ff, 220 USPQ at 938. In the absence of such special findings and of a proper charge on which a conclusion of obviousness can be reviewed, and because the parties here are not yet in substantial agreement as to facts bearing on the obviousness issue, it is appropriate to remand the present case.

[14] Accordingly, in light of the above errors, we cannot affirm the lower court's denial of Envirotech's JNOV motion regarding invalidity. "(This) court must be satisfied *** that the party challenging validity has [properly] carried its burden of overcoming the presumption (of validity)." *Medtronic, Inc. v. Cardiac Pacemakers, Inc.*, 721 F.2d 1563, 1567, 220 USPQ 97, 100 (Fed. Cir. 1983). We are not satisfied. As a court never "declares" a patent valid, we merely vacate and remand on the issue of validity. We leave initially to the district court the extent to which a new trial is required if the issue of patent invalidity is pursued.¹¹ However, if the parties can stipulate all the relevant facts, the judge can decide for himself the legal issues relating to validity. See *White v. Jeffrey Mining Machinery Co.*, 723 F.2d 1553, 220 USPQ 703 (Fed. Cir. 1983).

Conclusion

We affirm the judgment that the inventions set forth in claims 1, 14 and 15 of the '210 patent, and in claims 1, 10, 11 and 12 of the '706 patent are not infringed; we vacate and

¹⁰ Interrogatory No. 2 pertaining to obviousness read: "Do you find that the defendants have proved by clear and convincing evidence that the differences between the patent claims and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains?" Answer: "Was obvious". That 'interrogatory' appears to be an inapt instruction and the jury's answer a type of special verdict on the issue of obviousness.

¹¹ It a new jury trial including obviousness is had, it would be preferable to submit at least detailed special interrogatories as to the facts enumerated in *Graham v. John Deere*, supra; *Connell v. Sears, Roebuck & Co.*, supra; *American Hoist & Derrick Co. v. Sowa & Sons, Inc.*, supra.

⁹ We do not, at this time, pass on whether the amendment was properly allowed.

remand on the issue of the validity of these claims. Each side will bear its own costs in this court.

Affirmed in Part, Vacated and Remanded in Part.

Baldwin, Circuit Judge, specially concurring.

I only wish to clarify that in this case the claims at issue were submitted to the jury with proper instructions. The jury in its deliberations construed the claims in accordance with those instructions and then determined there was no infringement.

Further, regarding the '210 patent, we have not construed claim 1 de novo in this appeal. We are presuming this particular construction as it is consistent with the jury verdict and it is supported by substantial evidence.

Court of Appeals, Federal Circuit

*Lindemann Maschinenfabrik GMBH
v. American Hoist and Derrick Company et al.*

No 83-1178

Decided Mar. 21, 1984

PATENTS

1. Patentability — Invention — In general (§51.501)

Pleading and practice in courts — Issues determined — In general (§53.501)

Statement by district court — "But I am not certain in my own mind at this point whether or not these gentlemen on the '315 patent invented anything." reflects misconception of role of courts under 35 USC 103; question mandated by statute is not "invention," but patentability; moreover, court's role in relation to patentability does not require it to conclude whether something was or was not "invented," or whether court subjectively considers invention worthy of patent protection; court's role is actually more simple; under statute, it is to determine whether patent's challenger carried burden of establishing invalidity.

2. Patentability — Anticipation — In general (§51.201)

Anticipation is factual determination, reviewable under the "clearly erroneous"

standard; anticipation requires presence in single prior art reference disclosure of each and every element of claimed invention, arranged as in claim; in deciding issue of anticipation, trier of fact must identify elements of claims, determine their meaning in light of specification and prosecution history, and identify corresponding elements disclosed in allegedly anticipating reference.

3. Pleadings and practice in courts — Burden of proof — Validity (§53.138)

Presumption from patent grant — In general (§55.1)

Statutory presumption of patent validity cannot "vanish" or be "weakened" and statutorily assigned burden of proof cannot be shifted; at same time, much confusion can be avoided by patentees who refrain from efforts to expand role of presumption beyond its burden-assigning and decisional approach-governing function; burden upon challenger of validity under 35 USC 282 is to introduce evidence of facts establishing invalidity, thus overcoming presumption; such evidence, if it is to carry the day, must be clear and convincing; because mere introduction of non-considered art, common phenomenon, does not "weaken" or otherwise affect presumption, there is no basis for adjusting required level of proof downward to "mere preponderance;" that clear and convincing standard may more easily be met when such non-considered art is more pertinent than cited art means that determination of whether patent challenger has met burden turns on relationship of uncited art to claimed invention.

4. Presumption from patent grant — Patent Office consideration of prior art (§55.5)

District court's view that "the 'Field of Search' is exactly what it purports to be and nothing more, that 'References Cited' are patents found within field which were actually considered by the examiner and listed because he found them to be most relevant," is flawed; examiner could not determine which patents are "most relevant" without considering number which are less relevant.

5. Pleading and practice in courts — Burden of proof — Validity (§53.138)

Presumption from patent grant — Patent Office consideration of prior art (§55.5)

Because touchstone is whether uncited art is sufficiently more relevant than that cited to serve as evidence of obviousness, argument respecting presumption based on uncited art's

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